CHAPTER 13

QUATERNARY AMMONIUM ANTIMICROBIAL COMPOUNDS

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The quaternary nitrogen moiety is an essential component for many biologically active compounds. Quaternary ammonium compounds play an important role in the living process. From vitamins (vitamin B complex and thiamine) to enzymes carboxylase, which participates in the carbohydrate metabolism, to choline, which is involved in transmethylation reaction of the fat metabolism, and to acetylcholine, a mediator in the transmission of nerve impulses (Burger, 1960), all play a fundamental function.

There are at least four types of physiologic actions (Burger, 1960) associated with quaternary ammonium compounds: (1) Curare-like (curaremimetic or curareform) action, a muscular paralysis with no involvement of central nervous system or heart, produced by d-tubocurarine chloride used to induce muscular relaxation during surgery; (2) muscarinic-nicotinic action, which is a direct stimulation of smooth muscles, and is a primary transient stimulation and secondary persistent depression of sympathetic and parasympathetic ganglia; (3) ganglia-blocking action; and (4) neuromuscular blockade. Table 13–1 illustrates the chemical structures of representative compounds responsible for these physiologic actions.

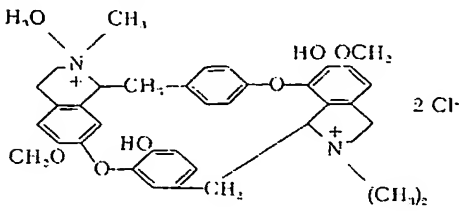
Medicinal chemists using the principle of structure activity relationships (Goldstein, 1974) have synthesized many quaternary ammonium compounds that will mimic certain biologic effects. Thus a very complex structure of d-tubocurarine chloride can be reduced to a much simpler decamethonium structure, a neuromuscular blocking agent. Hexamethonium acts as a ganglionic blocker by preventing the receptor from responding to acetylcholine. The decamethonium is too long to fit the ganglionic receptor but would act as a neuromuscular blocker by preventing the combination of acetylcholine with muscle end plate receptors. When the number of carbon atoms separating the quaternary nitrogens is increased above 12 carbons, the autonomic nervous activity disappears and the compounds become surface active and antimicrobial. However, there are exceptions to this rule, as we will discuss later on in the case of bis-quaternary and polymeric quaternary ammonium compounds, where two and four carbon atoms separate the quaternary nitrogen, and the products have antimicrobial properties. This chapter is concerned exclusively with antimicrobial quaternary ammonium compounds.

DISCUSSION

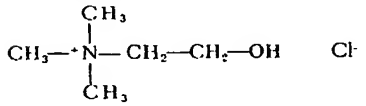
Although there are many stages in the historical development of quaternary ammonium germicides, there is general agreement on at least two truly historical milestones. The first is the work of Jacobs et al., which examined structure, preparation, and antimicrobial activity. Jacobs and Heidelberger, in 1915, published a number of papers describing the preparation of various different series of the quaternary ammonium salts of hexamethylenetetramine. In 1916 Jacobs and his coworkers published three papers describing the antimicrobial activity of many of the quaternary ammonium compounds they had previously synthesized and of additional derivatives prepared therefrom. In these publications, they related structure to antimicrobial activity. Although some reviews have challenged the work of Jacobs et al. as the earliest investigations of quaternary ammonium germicides, their preeminence is assured by their quality, quantity, and treatment, which included antimicrobial activity and correlation between structure and antimicrobial activity. The only valid criticism of the work of Jacobs may be the fact that some of the antimicrobial activity of his products may be due to the release of formaldehyde from hexamethylenetetramine. Jacobs reported that the antimicrobial activity is due to the presence of hexamethylenetetramine nucleus. Methenamine mandelate USP is used as a urinary tract anti-infective

Table 13–1. *Quaternary Structures with Physiological Actions*

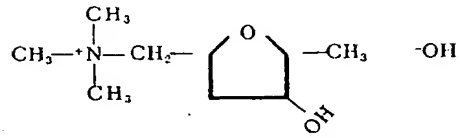
[1] d-Tubocurarine Chloride USP



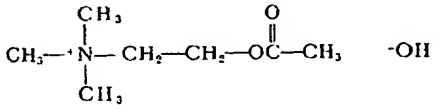
[2] Choline Chloride



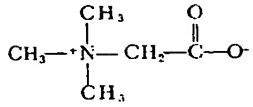
[3] Muscarine Hydroxide



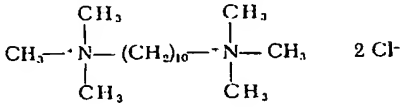
[4] Acetylcholine Hydroxide



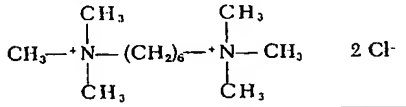
[5] Betaine



[6] Decamethonium Chloride



[7] Hexamethonium Chloride



even today, acting by releasing formaldehyde in an acid medium (Gennaro, 1985).

In any consideration of earlier investigations, the reader is reminded that our interests are solely with the various aspects of the antimicrobial activity of the quaternary ammonium compounds. Consequently, we may exclude those early publications dealing completely with synthesis of these compounds and in addition refute the role attributed by other reviews to the nineteenth century research on curare (Cucci, 1949).

Not until the 1920s did any additional information appear, at which time Browning et al. (1922, 1926) and Browning (1926) described the bacterial activity of quaternary derivatives of pyridine, quinoline, and other ring structures. Hartmann and Kagi, in 1928, reported on the antibacterial activity of quaternary ammonium compounds of acylated alkylene diamines. It was not until 1935, when Domagk disclosed the antibacterial activity of the long-chain quaternary ammonium salts, that the second and most important milestone in the development of antimicrobial quaternary ammonium compounds took place. The improved germicidal activity that occurred when a large aliphatic residue was attached to the quaternary nitrogen atom established the practicability and utility of these compounds, first in medicine and later in many other applications. This important disclosure stimulated research in synthesis and antimicrobial testing of quaternary ammonium compounds, with the consequent frequent publications and patents issued from 1935 to the present. After Domagk's discovery of the biocidal properties of cationic surface active agents several generations of structurally variable quaternary ammonium antimicrobials of commercial importance were developed.

The first generation is the standard benzalkonium chloride of specific alkyl distribution, namely C_{12}—40%, C_{14}—50%, C_{16}—10%. Another version of equally commercially successful alkyl distribution in the benzalkonium series is C_{12}—5%, C_{14}—60%, C_{16}—30%, C_{18}—5% as shown in Table 13–2. The official United States Pharmacopeia recognizes the benzalkonium chloride as a pharmaceutical aid (antimicrobial preservative). The USP specification for the C_{12}/C_{14} homologs components is 70% minimum of the total alkylbenzyldimethyl ammonium chloride content. This broad specification does not always give the most efficacious product. The major determining factor for biocidal efficacy is the hydrophilic-lipophilic balance of the products. The peak for biocidal activity of the homolog series is illustrated on Table 13–3, with a carbon chain of 14 offering the best activity (Cutler et al., 1966; Daoud et al., 1983; Hansch et al., 1973, 1964; Lien et al., 1976, 1968).

Modifications in the first-generation quaternaries by substitution of the aromatic ring hydrogen with chlorine, methyl, and ethyl groups resulted in the second generation of the substituted benzalkonium compounds. Out

Table 13–2. *Commercial Antimicrobial Quaternary Ammonium Compounds*

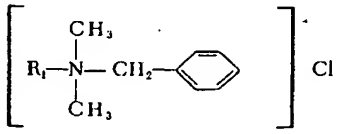
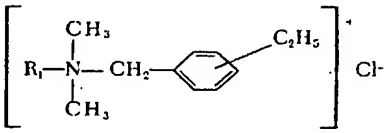
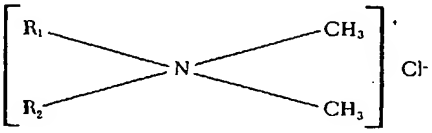
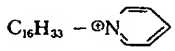
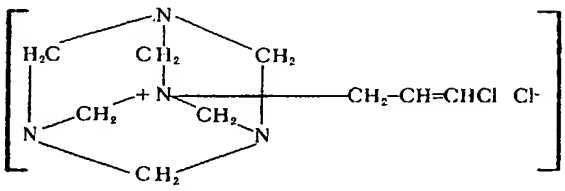
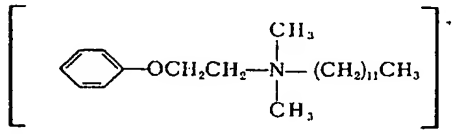
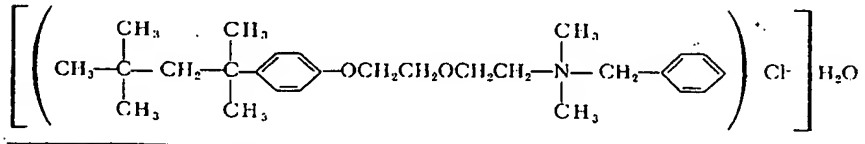
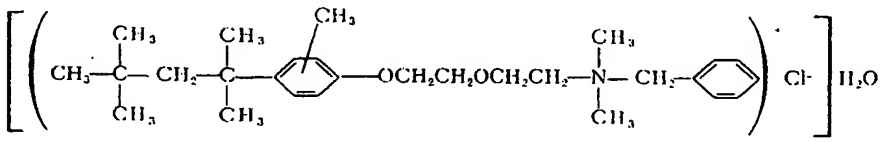
Chemical Structure	*Trade Names*	*Manufacturers*
Benzalkonium Chlorides:		
	BTC 835, BTC 824	Stepan (Onyx)
	BTC 50 USP	"
	Barquat MB-50	Lonza
	Variquat 50 MC	Sherex
	Zephiran Chloride	Winthrop
	Hyamine 3500	Lonza
R_1 = C_{12}—40%, C_{14}—50%, C_{16}—10%	Maquat MC 1412	Mason
R_1 = C_{12}—5%, C_{14}—60%, C_{16}—30%, C_{18}—5%	Maquat MC 1416	Mason
Substituted Benzalkonium Chlorides:*		
	BTC 471	Stepan (Onyx)
	BTC 2125M	"
	Barquat 4250	Lonza
	Maquat MQ-2525M	Mason
R_2 = C_{12}—50%, C_{14}—30%, C_{16}—17%, C_{18}—3%		
R_2 = C_{12}—68%, C_{14}—32%		
*See Text and Brochures		
Twin Chain Quaternaries:		
	BTC 818, BTC 812	Stepan (Onyx)
	BTC 1010	"
	Bardac 2050	Lonza
	Bardac 205M	"
	Bardac 2250	"
Dioctyl 25%, Didecyl 25%, Octyldecyl 50%		
R_1 = Octyl. R_2 = Dodecyl		
Cetylpyridinium Chloride:		
	Cepacol Chloride	Merrell Labs.
	Ceepryn Chloride	"
N-(3-Chloroallyl)Hexaminium Chloride:		
	Dowicide Q	Dow
	Dowicil 200	Dow
	Dowicil 75	Dow
Domiphen Bromide:		
	Bradosol	Proctor & Gamble
	Oradol	Ciba
	Modicare	"
Benzethonium Chloride:		
	Phemerol Chloride	Parke-Davis
	Hyamine 1622	Rohm & Haas
		Lonza
Methylbenzethonium Chloride:		
	Diaparene Chloride	Rohm & Haas
	Hyamine 10X	Rohm & Haas

Table 13–3. *Effect of Length of Carbon Chain on Bactericidal Activity of Benzalkonium Chloride*

	Bactericidal Test (Minimum Concentration That Kills in 10 Minutes but Not in 5 Minutes in ppm)		
Long-Chain Length	*Staphylococcus aureus #6538*	*Salmonella typhosa #6539*	*Pseudomonas aeruginosa #15,442*
8	3000	4500	6000
9	800	1400	2500
10	450	300	1200
11	160	130	400
12	45	40	120
13	25	20	50
14	15	12	40
15	25	20	70
16	30	25	200
17	170	15	360
18	450	60	1000
19	330	90	1300

of this group the product with commercial significance is the alkyldimethylethylbenzyl ammonium chloride under the trade name BTC 471 with alkyl distribution C_{12}—50%, C_{14}—30%, C_{16}—17%, C_{18}—3%. Another product with high biocidal activity in this group is alkyldimethyl-3,4-dichlorobenzyl ammonium chloride under the trade names of Tetrosan 3,4D, and Riseptin with same alkyl distribution as above.

By far the product of greatest commercial significance today is of the third generation of quaternary ammonium compounds, the *dual quats*, developed in 1955 under the trade name BTC 2125M. This product is a mixture of equal proportions of alkyldimethylbenzyl ammonium and alkyldimethylethylbenzyl ammonium chlorides of specific alkyl distribution, as shown in Table 13–2.

This combination of benzalkonium chloride with alkyl distribution (C_{12}—5%, C_{14}—60%, C_{16}—30%, C_{18}—5%) and alkyldimethylethylbenzyl ammonium chloride with alkyl distribution (C_{12}—68%, C_{14}—32%) is BTC 2125M, with superior microbiologic performance (Onyx or Stepan brochures). This synergistic combination of the third generation of quaternaries not only had an increased biocidal activity, it reduced the acute oral LD_{50} from 0.3 g/kg of benzalkonium chloride to an acute oral LD_{50} of 0.750 g/kg for BTC 2125M.

In summary, the third generation—the dual quats—offer improved biocidal activity, stronger detergency, and a relatively lower level of toxicity. In the early 1950s the nonionic detergents were being developed with far greater cleaning power than natural soaps. The compatibility of quaternary ammonium compounds with nonionic detergents resulted in superior formulations that helped overcome the environmental factors, such as hard water, anionic residues of soap, and proteinaceous soils, which were found to weaken their effectiveness (Greene and Petrocci, 1980).

A continual change and improvement in advancing and broadening the spectrum of biocidal activity enabled disinfectants to work under the most adverse conditions and produced safer, more economical products. In 1965 another technological development, catalytic amination of long-chain alcohols, made commercially feasible the production of dialkylmethyl amines, which in turn can be quaternized with methyl chloride to give us the *twin chain quats*, the fourth generation of quaternaries antimicrobials with high performance, unusual properties, and tolerances (Ditoro, 1969; Petrocci et al., 1974). The twin chain quats, like dioctyl dimethyl ammonium bromide and didecyldimethyl ammonium bromide were first introduced by the British Hydrological Corporation (BHC, 1947. "DECIQUAM 222") for the British food industry.

These products displayed outstanding germicidal performance; unusual tolerance for anionic surfactants, protein loads, and hard water; and even low-foaming characteristics. Table 13–4 illustrates the cidal activities and pseudomonicidal, fungicidal, and hard-water tolerance of five of the most active twin chain quats, out of 20 as measured by the official AOAC procedure reported by Petrocci et al. (1974). The product of choice in this series is C_8/C_{12}DMAC because of its superior water solubility and germicidal activity. However, the odd number chain C_9/C_{11}DMAC is an equally active product, although its commercial feasibility has not been explored because of high cost of the odd carbon chain alcohols. Recently several odd carbon chain alcohols have been offered in semicommercial quantities. The concept of synergistic

Table 13–4. *Antimicrobial Activity of Twin-Chain Quaternary Ammonium Compounds*

Compounds	*Pseudomonicidal*	*Fungicidal*	*HWT*
1. C_{10}/C_{10}DMAC	500 PPM	210 PPM	1100 PPM
2. C_8/C_{12}DMAC	500 PPM	200 PPM	1200 PPM
3. C_9/C_{11}DMAC	500 PPM	190 PPM	1400 PPM
4. C_8/C_{12}DMAC	550 PPM	235 PPM	1300 PPM
5. C_{10}/C_{11}DMAC	550 PPM	210 PPM	1300 PPM

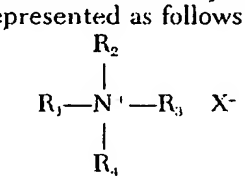
HWT = Hard Water Tolerance; DMAC = Dimethylammonium Chloride

combination in the dual quats has been applied to twin chain quats dialkyldimethyl ammonium chlorides (dioctyl—25%, didecyl—25%, octyldecyl—50%) was combined with benzalkonium chlorides (R=C_{12}—40%, C_{14}—50%, C_{16}—10%). According to the work of Schaeufele (1984), a 60/40 blend of the above quaternaries proved to be superior to the individual components tested via the AOAC Use-Dilution Test (Official Methods of Analysis of the AOAC, 1984). This newest blend of quaternaries represents the fifth generation of quaternary ammonium compounds. The blend remained active under the most hostile conditions, was less toxic and less costly, and provided more convenient disinfectants.

In the 1980s the toxicity of quaternaries underwent scrutiny by the EPA and other U.S. regulatory agencies. The safety of the biocides in general received top priority over their efficacy. A new class of biocides, the polymeric quaternaries, has emerged, which are less toxic than the standard benzalkonium chlorides and less powerful than the dual quats or twin chain quats. The polymeric quaternaries are milder and have found applications in pharmaceuticals as preservatives (Stark, 1985; Merianos, 1988). The polymeric quaternary ammoniums are polyelectrolytes, representing the sixth generation of quaternary ammonium compounds. They are milder and safer than all other classes based on LD_{50} and cytotoxicity (Stark, 1985). More recently another synergistic combination, which may be considered the seventh generation of quaternary blends of bis-quats and polymeric quaternary (Polyionenes), offer excellent antimicrobial activity against oral flora bacteria, namely *Bacteroids gingivalis* 318, A7A128, *Actinomyces viscosus*, and *Streptococcus mutans* in the level of 1 to 5 ppm in a pharmaceutically accepted polymer formulation (MerPan Chemical Consultants Brochures, MerPanocide L. and MerPanocide LHD) (Merianos, 1988). Reduction of toxicity in the bis-quats without compromising efficacy is not an easy task. These preliminary results required further testing to confirm safety and true synergism in this series of polymeric quaternaries with bis-quats blends (Merianos, 1986).

CHEMISTRY

The quaternary ammonium compounds are the products of a nucleophilic substitution reaction of alkyl halides with tertiary amines. Chemically, they have four carbon atoms linked directly to the nitrogen atom through covalent bonds, while the anion in the original alkylating agent becomes linked to the nitrogen by an electrovalent bond. The general formula for the quaternary ammonium compounds is represented as follows:



R_1, R_2, R_3, and R_4 are alkyl groups that may be alike or different, substituted or unsubstituted, saturated or unsaturated, branched or unbranched, and cyclic or acyclic, and that may contain ether or ester or amide linkages; they may be aromatic or substituted aromatic groups. The nitrogen atom plus the attached alkyl groups forms the positively-charged cation portion, which is the functional part of the molecule. The portion attached to the nitrogen by an electrovalent bond may be any anion, but is usually chloride or bromide to form the salt.

Depending on the nature of the R groups, the anion, and the number of quaternary nitrogen atoms present, the antimicrobial quaternary ammonium compounds may be classified as follows.

Monoalkyltrimethyl Ammonium Salts

In this instance, one R group is a long-chain alkyl group, and the remaining R groups are short-chain alkyl groups, such as methyl or ethyl groups. All of the quaternary compounds in this group are prepared from the reaction of a tertiary amine with an alkyl halide (Domagk, 1935; Shelton et al., 1946; Hartmann, 1929). The tertiary amine may be the long-chain alkyldimethylamine or the short-chain trimethylamine, which react with methyl halide or with the long-chain alkyl halide, respectively. Examples of commercially available products in this group are cetyltrimethylammonium bromide as CTAB, alkyltrimethyl ammonium chloride as Arquad 16, alkylaryltrimethyl ammonium chloride as Gloquat C, cetyldimethyl, ethylammonium bromide as Cycloton D256B, Ammonyx DME, and Bretol.

Monoalkyldimethylbenzyl Ammonium Salts

In this group, one R is a long-chain alkyl group, a second R is a benzyl radical, and the two remaining R groups are short-chain alkyl groups, such as methyl or ethyl groups. These compounds are prepared by the reaction of a long-chain alkyldimethylamine with the benzyl halide (Wakeman and Tesoro, 1954; Domagk, 1938; Dunn, 1936).

Examples of commercially available products in this group are alkyldimethylbenzyl ammonium chlorides, as BTC 824, Hyamine 3500, Cyncal Type 14, and Catigene.

In addition, there are substituted benzyl quaternary ammonium compounds such as dodecyldimethyl-3,4-dichlorobenzyl ammonium chloride, sold under the trade name of Riseptin. There are also mixtures of alkyldimethylbenzyl and alkyldimethyl substituted benzyl (ethylbenzyl) ammonium chlorides, such as BTC 2125M, Barquat 4250 (Stepan; Lonza Brochures).

Dialkyldimethyl Ammonium Salts

In this instance, two R groups are long-chain alkyl groups, and the remaining R groups are short-chain alkyl groups, such as methyl groups. These compounds are prepared by the reaction of the long-chain alkyldimethylamine with a long-chain alkyl halide or dialkylmethylamine with methyl halide (Kirby and Frick, 1963; Tonaka, 1944; Kuhn et al., 1940).

Examples of commercially available products in this

group are didecyldimethyl ammonium halides, such as Deciquam 222 and Bardac 22, and octyldodecyldimethyl ammonium chloride, such as BTC 812 (Stepan; Lonza Brochures).

Heteroaromatic Ammonium Salts.

In this group, one R chain is a long alkyl group, and the remaining three R groups are provided by some aromatic system. Thus, the quaternary nitrogen to which these three R groups are attached is part of an aromatic system such as pyridine, quinoline, or isoquinoline.

These compounds are prepared by reaction of the aromatic amine with a long-chain alkyl halide (Browning et al., 1922; Shelton et al., 1946; Mosher and Howard, 1948). Examples of commercially available products in this group are cetylpyridinium halide (CPC and Ceepryn), reaction product of hexamethylenetetramine with 1,3-dichloropropene to give cis-isomer 1-[3-chloroallyl]-3,5,7-triaza-1-azoniaadamantane (Dowicil 200), alkylisoquinolinium bromide (Isothan Q), and alkyldimethylnaphthylmethyl ammonium chloride (BTC 1100).

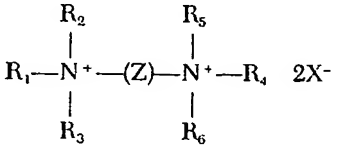
Polysubstituted Quaternary Ammonium Salts

In this group, the cation portion of the molecule is the same as that described for any of the aforementioned groups. However, the anion portion is not a small inorganic ion as previously described, but a large, high-molecular-weight organic ion. These compounds are prepared by reaction of the quaternary ammonium halides with the sodium, potassium, or calcium salt of a high-molecular-weight organic moiety, so that an exchange of the anions is effected (Wakeman et al., 1971; Shibe et al., 1955).

Examples of commercially available products in this group are alkyldimethylbenzyl ammonium saccharinate (Onyxide 3300 and Loroquat QA 100), and alkyldimethylethylbenzyl ammonium cyclohexylsulfamate (Onyxide 172).

Bis-Quaternary Ammonium Salts

In this group of compounds, there are two symmetric quaternary ammonium moieties arranged in the general formula:



Here the R groups are as described for any of the aforementioned groups, Z is a carbon-hydrogen chain, and an anion is attached to each quaternary nitrogen via an electrovalent bond. These compounds are prepared by reaction of a bis-tertiary amine with alkyl halide or of a di-halo compound with a tertiary amine (Hwa, 1963; DeBenneville and Bock, 1950; Babbs et al., 1956).

An example of a commercially available product in this group is 1,10-bis(2-methyl-4-aminoquinolinium chloride)-decane, sold under the trade name of Dequadin or Sorot. Another example of bis-quat is 1,6-Bis [1-methyl-3-(2,2,6-trimethyl cyclohexyl)-propyldimethylammonium chloride] hexane or triclobisonium chloride sold by trade name Triburon. Another commercially available bis-quat is CDQ (Buckman Brochures), used for industrial water treatment for controlling sulfate-reducing bacteria (*Desulfovibrio* sp.). The CDQ is prepared by reaction of alkyl[C_{12}—40%, C_{14}—50%, C_{16}—10%] dimethylamine with dichloroethyl ether. Also, reaction of 1,4-dichloro-2-butene with two moles of alkyldimethylamines or hexamethylenetetramine offer another example of bis-quats with broad spectrums of biocidal activity.

Polymeric Quaternary Ammonium Salts

Many different types of polymeric quaternary ammonium salts have been reported to have antimicrobial activity (Ghosh, 1988, 1986; Ikeda and Tazuke, 1983, 1984, 1985; Samour, 1978; Rembaum, 1973, 1975, 1977). The methods of preparation of these polymers are also many, from free radical polymerization of monomers containing quaternized nitrogen, to cationic, anionic polymerization, polycondensation of diamines with dihalides, or polycondensation of haloamines. The last method was used by Rembaum for the preparation of ionenes, which are polyelectrolytes with positively-charged nitrogen atoms located in the backbone of polymeric chain. This type of polycation was first reported in 1941 and is formed by the Menchutkin reaction from ditertiary amines and dihalides (Rembaum, 1973). Although a number of patents were published since 1941 concerning the applications of ionene polymers (Fush and Stamberger, 1965; Boyer and Santiago, 1974; Walker and Cambre, 1970), little information was available on the mechanism and kinetics of their formation, or their solution properties. The scope of this polycondensation reaction, the effect of concentration and solvent on rates and molecular weight of ionenes, and a proposed mechanism were first reported by Rembaum in 1968, when it was realized that under well defined conditions relatively high molecular weight (3,3 ionene chloride 65,000) ionenes were obtainable. The polymerization conditions for the formation of ionenes involves a total concentration of 3.0 mol [1.5 mol of each monomer] in a mixture of DMF/MEOH solvent [1:1 or 4:1] and 5 to 7 days of reaction time at room temperature to give polyionenes with molecular weight of about 65,000. In condensation polymerization the purity of the condensing species and stoichiometry are critical in obtaining high-molecular-weight polymer. Rembaum (1973) reported that polyionenes exhibit the following biologic effects: (1) *Bactericidal action*, (2) *Formation of insoluble complexes with DNA and heparin*, (3) *Neuromuscular blocking action*, (4) *Cell lysis and aggregation*, and (5) *Cell adhesion*. The antimicrobial and antifungal properties of ionenes were studied by the zone of inhibition method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida sporogenes, S. typhimurium, M. smegmatis, Pseudomonas mirabilis, Pseudomonas vulgaris,*

Table 13–5. *Minimum Inhibitory Concentration of Polyionenes*

Polyionene	MIC (ppm)	
	S. aureus	*E. coli*
1. 3, 3-ionene bromide	>128	>128
2. 6, 6-ionene bromide	16	16
3. 6, 10-ionene bromide	4	4
4. 2, 10-ionene bromide	4	8
5. 6, 16-ionene bromide	4	32

Candida globosum, M. verrucaris, F. oxysporum and *Alternaria sp.* The MIC for five of the most active ionenes bromides against *S. aureus* and *E. coli* are listed in Table 13–5.

The 6,10 ionene bromide is the most active in these series, and at concentrations up to 4 ppm stimulated the growth of normal cells while this range showed inhibition and death of the transformed human cells W138, possibly by electrostatic cytotoxic interaction (Rembaum et al., 1975). It has been suggested that malignant cells are more electronegative than normal cells. If so, malignant cells should demonstrate a greater affinity for the electropositive ionene polymer. This hypothesis has been supported by experimental work. However, this preliminary study appears to warrant more extensive studies to evaluate this class of polymers as chemotherapeutic agents. Rembaum concluded that polyionenes and their low-molecular-weight analogues constitute a unique model system for a molecular probe of the living cell machinery, because their structure, their positive charge densities, their counterions, and their molecular weights can be varied systematically. These considerations apply not only to the study of toxicity or antimicrobial activity, but also to the understanding of the interaction of the polyionenes with DNA or as molecular probes to elucidate the properties of cell membranes.

The high charge density of polyionenes is responsible for their bactericidal and fungicidal activities, for the prolonged duration of the curarizing action, and for the formation of complexes with DNA and heparin. The differences in the biologic properties or stability of the complexes may be explained on the basis of electrostatic association between the negative and the positive moieties of the interacting molecules.

Because the normal and neoplastic cells show an array of different surface properties, including an increase in anodic mobility after transformation, some polyionenes (e.g., 6,10 ionene bromide) may preferentially bind to cancer cells and inactivate them. This differential binding and toxicity to transformed cells conforms with topologic changes of membrane binding sites in the transformed cells. All four products in Table 13–6 are ionenes containing the quaternary nitrogen atom on the backbone of the polymer chain. The main difference is the molecular weight, which is due to reactivity of the alkylating monomer, reaction time, solvent, temperature, and method of preparation. The ionene bromides (MW 65,000) reported by Rembaum, however, are not commercially available, despite their many biologic effects and potential applications, perhaps due to poor yields, incomplete reaction (65 to 75% conversion), long reaction time, and toxicity. The other three products listed in Table 13–6 are items of commerce. The WSCP or Busan 77 from Buckman Laboratories, which is chemically identified as poly[oxyethylene(dimethyliminio)-ethylene (dimethyliminio)-ethylenedichloride] is made by condensation of N,N″ tetramethylethylenediamine with dichloroethyl ether in water to give molecular weight 2000 to 3000. The molecular weight was measured by Gel Permeation Chromatography (GPC) using polyethyleneglycol standard according to Levy and Dubin's reported method (Merianos, 1986).

The manufacturer has provided brochures and data sheets describing minimum inhibitory concentration (MIC of 10 to 50 ppm) levels indicating a broad spectrum of antimicrobial activity, with dual functionality as microbicide and polymer clarifier for swimming pools. The material is registered with the Environmental Protection Agency as a swimming-pool algicide, with 2 ppm of active product used as a maintenance dose, and 5 to 8 ppm of active product used to rid pools of heavy, objectionable algae growth. The presence of WSCP at only 2 ppm can reduce by 80% or more the chlorine needed to kill the bacteria. In general, WSCP enhances the activity of all oxidizers used in the swimming pools. In addition, the material is registered with the Environmental Protection Agency as a cooling water treatment biocide at 20 to 40 ppm of active product. WSCP microbicide formulations can be used to control the growth of algae, bacteria, and fungi on cooling towers and other parts of commercial and industrial recirculating cooling water systems.

Another polymeric quaternary ammonium compound is the product Mirapol A-15 from the Miranol Chemical Company, which is chemically identified as poly[N-3-dimethylammonio)propyl]N-[3-ethyleneoxyethylenedimethylammonio)propyl]urea dichloride] or by the CTFA name Polyquaternium 2. This product is made by reacting a symmetrically substituted urea ditertiary amine with dichloroethylether in water to give MW 2000 to 3000.

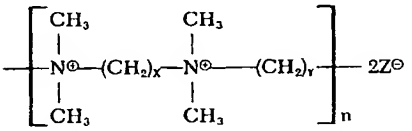
The manufacturer, who is primarily concerned with hair care products, reports a static level of 80 ppm and a destruction level of 100 ppm against *Pityrosporum ovale*. Additional examination of this structure demonstrates inhibitory levels at 100 ppm against both *Staphylococcus aureus* and *Escherichia coli*. By a time/kill water treatment screening procedure, the product demonstrates a high-percentage kill of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* at 10, 15, and 20 ppm of active product following a 30-minute contact period. Although unpublished reports by Petrocci have demonstrated antimicrobial activity for this product, attempts to repeat these results with current production of this product failed to confirm biocidal activity. This product

is not sold as an antimicrobial but as a hair conditioning agent for shampoos.

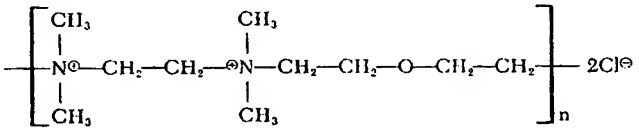
The last structure on Table 13–6 of the polymeric quaternary ammonium compound, from Onyx (Stepan) Chemical Company, is chemically identified as α-4-[1-tris(2-hydroxyethyl)ammonium chloride-2-butenyl] poly[1-dimethyl ammonium chloride-2-butenyl]-W-tris(2-hydroxyethyl)ammonium chloride, or Onamer M or Polyquat or with the CTFA name Polyquaternium 1. The Onamer M is made by reacting 1,4-bis[dimethylamino]-2-butene (0.9 mol), triethanolamine (0.2 mol), and 1,4-dichloro-2-butene (1.0 mol) in water to give an average molecular weight of 5000 to 10,000 (Green et al., 1975, 1977, 1978, 1982; Good et al., 1987). The purpose of the triethanolamine in the preparation was to randomly terminate the polymeric chain, so that we obtained a low-molecular-weight product by design. The 1,4-dichloro-2-butene alkylating agent is very reactive and gave us a 98% conversion of organic chloride to ionic within 6 to 8 hours in water. Polyquaternium 1 was originally intended for hair conditioning applications (Merianos et al., 1977); however, with the good antimicrobial activity combined with excellent toxicologic data, it emerges as an excellent preservative candidate for ophthalmic preparations (Stark, 1985). Today this product is registered with FDA as a preservative for contact lens solutions (Opti-Tears, Opti-Clean, Opti-Soft, Opti-Free, Polyflex Tears-Naturale II—all are trade names of Alcon Labs, Fort Worth, TX 76134). Table 13–7 lists the comparative cytotoxic response of Onamer M at various concentrations and other solutions by in-vitro testing of Mouse L929 cells (Stark, 1985). The most common ophthalmic preservatives are thimerosal, benzalkonium chloride, and chlorhexidine. These compounds are toxic to the eye and may cause corneal erosion and corneal ulceration resulting in pain. This problem is particularly severe with quaternary ammonium compounds that are concentrated more than 400 times by hydrophilic lenses. Chlorhexidine is concentrated as much as 100-fold by hydrophilic contact lenses, which results in the potential for injury to the eye. The comparative cellular toxicity of soft contact lenses soaked in Onamer M at various concentrations and other solutions was determined by in-vitro testing. Mouse L929 cells were grown

Table 13–6. *Polymeric Polyquaternary Ammonium Compounds*

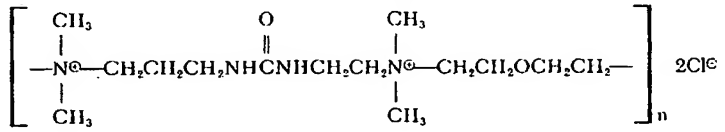
A. Ionenes A. Rembaum Applied Polymer Symposium No. 22 299-317 (1973)



B. Poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylene dichloride] (WSCP or Busan 77)

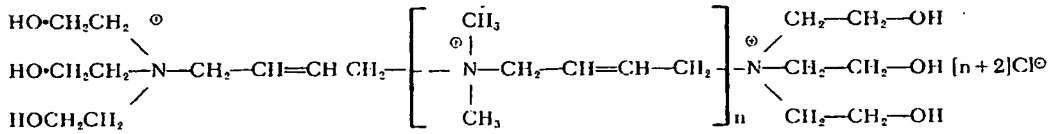


C. Polyquaternium 2 CTFA-adapted name (Mirapol-A15)



n = 6 (average)

D. Polyquaternium 1 CTFA-adapted name (Onamer M)



plus triethanolamine hydrochloride

Table 13–7. *Comparative Cytotoxicity of Soft Contact Lenses Soaked in Preserved Solutions on Mouse L929 Cells*

Lens Soaked	Cytotoxic Response: Cell Lysis	Cytotoxic Response: Zone of Cell Death (mm)	Cytotoxicity Conclusion†
Saline	–	0	None
0.001% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.1% Onamer M	–	0	None
0.3% Onamer M	–	0	None
1.0% Onamer M	+	31	Moderate
Alkyltriethanol ammonium* Chloride [0.03%] + thimerosal [0.002%]*	+	16	Minimal
Chlorhexidine [0.005%] + thimerosal [0.001%]*	+	25	Moderate
Thimerosal [0.001%]*	+	10	Minimal
Sorbic acid [0.1%]*	+	16	Minimal
0.01% Benzalkonium chloride	+	48	Severe
0.01% Benzalkonium chloride	+	64	Severe

*Commercially available marketed solutions.

†Cytotoxicity was rated as follows: Minimal zone of decoloration was 20 mm; Moderate zone of decoloration was 20–40 mm; Severe zone of decoloration was 40 mm.

on a basal salts medium using standard tissue culture techniques. The cells were grown until confluent growth was obtained. HEMA soft contact lenses were cycled through seven 8-hour cycles of fresh solution prior to exposure to the mouse cells. After soaking, the lenses were rinsed in water. The mouse cells were then exposed to each respective lens for 24 hours, whereupon the cell growth was examined microscopically and with staining procedures. The cytotoxic response cell lysis and zone of cell death was reported as in Table 13–7. The results from Table 13–7 conclusively show that Onamer M is not cytotoxic to Mouse L929 cells. This cytotoxic response may be related to the acute oral LD_{50} of these compounds listed on Table 13–8. Additional work is needed to prove if there is a correlation of cytotoxicity and acute oral LD_{50} of the biocides within the same class or different ones. This seems to be the case, if one compares Onamer M with the benzalkonium chloride and the other products. All are more toxic than Onamer M, as can be seen from LD_{50} reported in the literature. Petrocci et al. in 1979 examined this compound and determined that the product was bacteriostatic against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Streptococcus faecalis* at 50 ppm of active product.

Table 13–8. *Acute Oral LD_{50}: Commercial Antimicrobial Quaternary Ammonium Compounds*

Compounds	LD_{50}
1. Cetylpyridinium chloride	0.20 g/kg
2. Benzalkonium chloride	0.30 g/kg
3. Domiphen bromide	0.32 g/kg
4. Methylbenzethonium chloride	0.35 g/kg
5. Benzethonium chloride	0.42 g/kg
6. Didecyldimethylammonium chloride	0.53 g/kg
7. Octyldodecyldimethylammonium chloride	0.72 g/kg
8. BTC 2125M	0.75 g/kg
9. Dowicil 200	2.20 g/kg
10. 6,10-ionene bromide	1.00 g/kg
11. WSCP or Bussan 77	2.77 g/kg
12. Mirapol A-15 (not sold as antimicrobial)	2.85 g/kg
13. Onamer M or Polyquat or Polyquaternium-1	4.47 g/kg

Chem. Pharm. Bull., 34, 4215–4224.

In addition, Onamer-M, when tested by the EPA-required Use-Dilution method, demonstrated efficacy as a hard surface disinfectant at 300 ppm of active product against *Salmonella choleraesuis,* at 350 ppm of active product against *Staphylococcus aureus,* and at 800 ppm of active product against *Pseudomonas aeruginosa*. The product demonstrated excellent activity by a time/test at 10 and 15 ppm of active product against *Pseudomonas aeruginosa* and *Enterobacter aerogenes* following a 30-minute contact period.

At least two characteristics are common to the WSCP and Onamer-M polymeric quaternaries, making them uniquely different from the ordinary biocidal quaternary ammonium compound. One is the absence of foaming, even at high aqueous concentrations. The other is the remarkably low toxicity shared by these polymeric quaternary products. As an example, for Onamer-M at 30% active material, primary abraded and intact skin irritation scores were zero for all observation periods. Draize eye irritation studies recorded a mild conjunctival irritation with scores of 2 or 4 in each rabbit, which cleared on the second day of observation. The acute oral LD_{50} in rats was determined to be 4470 mg/kg. The product was described as nonmutagenic by the mouse lymphoma forward mutation assay and by the sex-linked recessive lethal test in *Drosphila melanogaster.* In addition, it was not considered carcinogenic by the in-vitro transformation of Balb/3T3 cells assay.

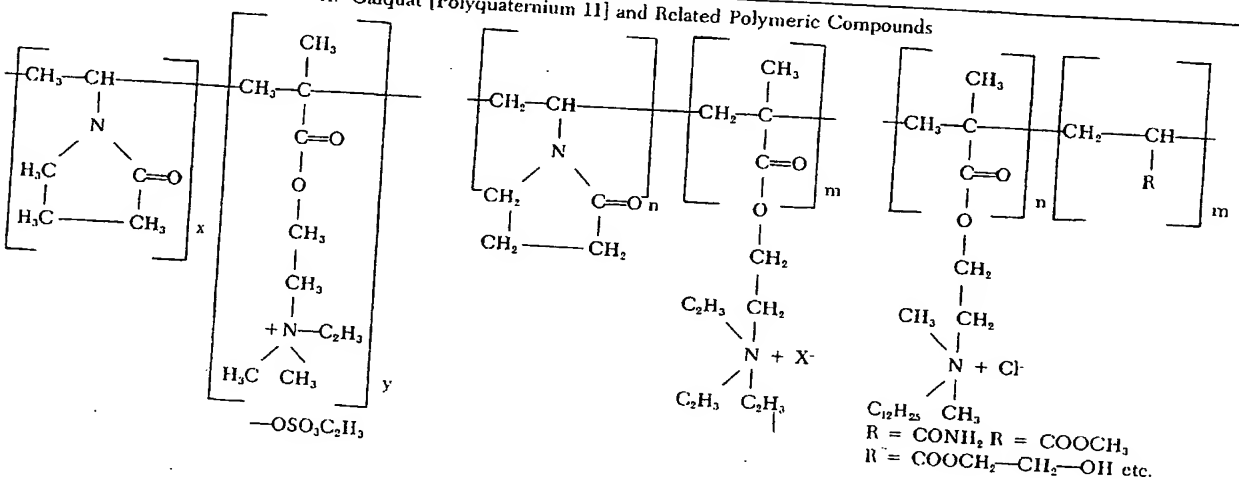
For reasons of structure, size, foaming, and toxicity, these polymeric quaternary compounds must be consid-

ered uniquely different from the biocidal quaternary ammonium compounds heretofore utilized. The results obtained to date should encourage synthesis within this structure group to produce additional compounds for consideration. Besides the polyionenes quaternary polymers there are other classes of antimicrobial polymers in which the quaternary nitrogen is pendant away from the backbone chain of the polymer (Table 13–9). Samour and coworkers (1978) reported a number of quaternary monomers, homopolymers, and copolymers of N-vinyl-pyrrolidone and 2-methacryloxyethyl-N,N,N-triethyl ammonium bromide and iodide with antimicrobial activity. This activity increases as the content of quaternary ammonium moiety increases. The quaternary nitrogen is pendant away from backbone chain and requires the proper lipophilic groups and proper charge density of the quaternary nitrogen for biocidal activity. In Table 13–9 are listed the most common polymers made by free radical polymerization. None of these polymers is used as an antimicrobial, but they are commercially available as hair conditioning agents for shampoos and other cosmetic products. Examples are Gafquats (Polyquaternium 11) and Merquats (Polyquaternium 7). These products are made by the free radical polymerization of diallyl quaternary monomer, and their molecular weight is over 100,000 (Butler et al., 1966—U.S. Patent No. 3,288,770).

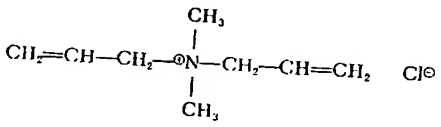
However, more recently Andrews et al. (1981—U.S. Patent No. 4,304,894) reported related Terpolymer, with molecular weight 10,000 to 20,000, to have surprisingly effective sterilizing activity against *Candida albicans* at 0.1%, when it was used as a preservative for soft contact lenses without accumulation into the lenses.

Table 13–9. *Free Radical Polymeric Quaternary Ammonium Compounds*

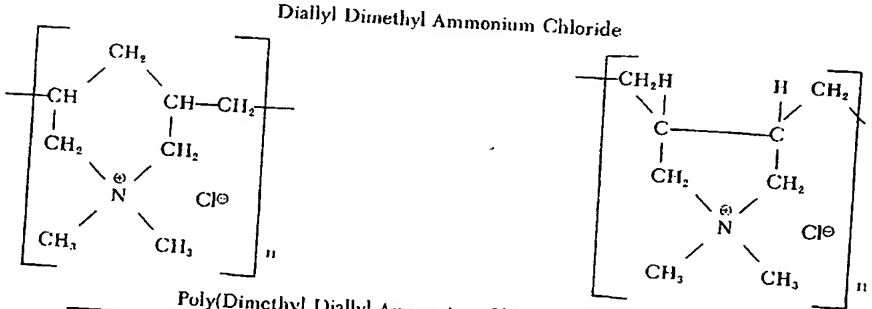
A. Gafquat [Polyquaternium 11] and Related Polymeric Compounds



B. Merquat (Polyquaternium 7)



Diallyl Dimethyl Ammonium Chloride



Poly(Dimethyl Diallyl Ammonium Chloride) (DMDAAC)

Ikeda and Tazuke (1983) reported that antimicrobial activities (MIC) of poly[trialkyl(vinylbenzyl)ammonium chloride] type of polycations against bacteria and fungi were more active than the corresponding monomers; however, the absolute activity is low compared to commercially used quaternaries. The antibacterial assessment was based only on the conventional spread plate method, which has been widely used to evaluate the antibacterial activity of ordinary antibiotics and disinfectants. However, the antibacterial activity of the polycations can not be determined precisely by this method on account of the adsorption of the polycations by some constituents in the culture media. The activity must be evaluated in the absence of any interfering material. More recently, Ikeda and Tazuke (1984, 1985) obtained peculiar results for poly[dodecyldimethyl{vinylbenzyl}ammonium chloride] and its monomer using the conventional spread plate method for evaluation. The MIC of the monomer was 10 to 100 ppm against *Escherichia coli*, *Aerobacter aerogenes* and *Pseudomonas aeruginosa*, whereas the MIC of the polymer product was greater than 1000 ppm against the same organisms.

The poly[dodecyldimethyl{vinylbenzyl}ammonium chloride interacted with the negatively charged species (such as sodium caseinate of the agar plate) and produced an insoluble complex, leading to inactivation of the polymer. In order to evaluate the antibacterial activity of such disinfectants correctly, it is preferred that the products come in contact with bacterial cells in sterile water or saline, and then that the surviving cells be counted after being cultivated on agar plates. Using this viable counting method the antibacterial activity of poly-[dodecyldimethyl{vinylbenzyl}ammonium chloride] was determined against *Staphylococcus aureus*, and at a concentration of 0.5 ppm all the bacterial cells were killed within 30 minutes of contact, whereas the corresponding monomer was inactive. Compounds with the longest alkyl chain studied (dodecyl) were found to exhibit high activity, and this was ascribed to the contribution of the increased hydrophobicity of the compounds to the activity.

In conclusion, the most significant finding was that the polymers are more active than the corresponding monomers, particularly against gram-positive bacteria. The higher activity of the polymers was interpreted as due to favored adsorption onto the bacterial cell surface and the cytoplasmic membrane with subsequent disruption of its integrity, although they have the disadvantage of diffusing through the cell wall, especially with the gram-negative bacteria.

Sheldon et al. (1985—U.S. Patent No. 4,532,128) reported that polymeric quaternary ammonium compounds having repeat vinylbenzylammonium units were antimicrobial and particularly useful for preserving ophthalmic solutions.

Ikeda and Tazuke in 1985 reported an optimal MW region of 14,300 for the 6,6-ionene bromide with antibacterial activity minimum bactericidal concentrations (MBC) of 6.6 ppm to 10 ppm against *Staphylococcus aureus*. The same workers reported that polymeric biguanides of MW 11,900 and poly{alkyl[C_2–C_{12}]-dimethyl[vinylbenzyl]ammonium chloride} of similar MW exhibit better bactericidal action against gram-positive than gram-negative bacteria. The poly{dodecyldimethyl[vinylbenzyl]ammonium chloride} was the most active with 0.5 ppm against *Staphylococcus aureus*, which suggests that hydrophobicity plays an important role in bactericidal action.

In conclusion, in all three types of polymers reported by Ikeda and Tazuke (1985), there is a clear MW dependence of the biocidal activity—that is, polymers with low MW as well as high MW exhibit lower bactericidal activity—and there exists an optimal MW region for the biocidal action. This MW dependence was confirmed in my work with Onamer M analogues (Merianos, 1986). It is my belief that a fine balance exists in the polymeric biocides between MW, biocidal activity (cytotoxicity), and (toxicity) acute oral LD_{50} as seen in Tables 13–8 and 13–7.

ANALYSIS

The chemical structure of quaternary ammonium compounds permits a variety of quantitative procedures to determine their concentration. Recently various analytical methods have been reported for the determination of benzalkonium chlorides by high-performance liquid chromatography (HPLC) (Marsh et al., 1983; Sato et al., 1984) and gas chromatography (GC) (Suzuki et al., 1989; Cybulski, 1984; Ng et al., 1986). Chemical ionization mass spectroscopy (MS) has been used to identify and determine the proportions of various alkyl chain lengths in commercial mixtures of benzalkonium chlorides (Dauod et al., 1983). The diphasic or the dye transfer method or the Epton method (1947) cannot be used for the determination of polymeric quaternary ammonium compounds. Recently, two methods for determination of low concentration of Onamer-M or polyquaternium 1 antimicrobial preservative in ophthalmic solutions (Good et al., 1987; Stevens and Eckardt, 1987) were reported. A direct titration technique for the accurate, quantitative determination of cationic and anionic polyelectrolytes has been reported by Wang and Shuster (1975). The technique is based on a direct neutralization reaction between cationic and anionic forms of the polymers. Modification of poly[vinylsulfuric acid] potassium salt (PVSAK) method has been used for determination of high concentration of Onamer-M (Merianos et al., 1977). The cationic polyelectrolytes show a light blue color in the presence of toluidine blue O dye, and the blue color turns to bluish purple when the titration end point is reached. With the many methods available, the method of choice is usually dictated by the level of quaternary ammonium compound anticipated in the sample. For solutions estimated to contain 0.5% or more quaternary ammonium compound, the chemist may employ a diphasic or direct titration procedure.

STRUCTURE-ACTIVITY RELATIONSHIPS

Jacobs and Heidelberger in 1916 were the first to report correlation between chemical structure and antimicrobial activity of benzyl and substituted benzyl hexamethylenetetrammonium salts. The results obtained with some 40 substances upon *Bacillus typhosus* demonstrated the existence of direct relationships between chemical constitution and bactericidal action within the series. The degree of the bactericidal action, however, is determined by the position, character, and number of groups substituted in the benzene nucleus. By introduction of the methyl, chlorine, bromine, iodine, cyano, and nitro groups into the benzene nucleus of the parent benzylhexamethylenetetrammonium salt, the bactericidal power of this compound was notably enhanced. The substitution of these groups in the ortho position almost invariably resulted in substances that were more active than their meta or para isomers; the introduction of the methoxy group was without marked effect.

Several substances in which two hexamethylenetetrammonium groups on the same aromatic nucleus (namely bis-quats 1,2-xylylenebis[hexamethylenetetrammonium chloride and mesitylenebis[hexamethylenetetrammonium chloride]) were found to be the most active of the substances of this series when tested against *Bacillus typhosus*. Comparative tests with other bacterial types demonstrated that these compounds possessed a marked degree of specificity for *Bacillus typhosus*. The bactericidal character is directly attributable to the presence of the hexamethylenetetramine nucleus.

The same general principle of increased antimicrobial activity was observed with benzalkonium and substituted benzalkonium chlorides, as illustrated in Table 13–10. The AOAC germicidal and detergent sanitizers test was used, and the hard water tolerance in ppm as $CaCO_3$ was measured. The test organism was *Escherichia coli* ATCC No. 11229; a 99.999% reduction in 30 seconds is required with quaternary level of 200 ppm to pass.

A synergistic blend of alkyl[C_{12}/C_{14} 70/30] 2,4,6-trimethylbenzyldimethyl ammonium chloride [C] has the highest hard water tolerance (HWT) of 1300 ppm, with the 2,4,5 isomer [D] having the next best HWT of 1200 ppm, which is twice as high as the corresponding benzalkonium chloride [A]. The mesitylenyl and pseudocuminyl quaternary compounds are highly crystalline nonhygroscopic materials with excellent microbiocidal properties; however, economics favors alkyl[C_{12}/C_{14} 70/30] ethyl[15% ortho, 85% para]benzyldimethylammonium chloride[B]. The HWT of the alkyl[C_{12} and C_{14}] ethylbenzyldimethylammonium chloride is reported in U.S. Patent Nos. 3,472,939 and 3,525,793.

Synergistic blends of microbiocidal quaternary ammonium compounds with dodecyl to tetradecyl proportion 85/15 to 55/45 are listed in Table 13–11.

Kourai and coworkers (1985) have reported on quantitative structure-activity relationships of antimicrobial N-laurylpyridinium iodides (Table 13–12). The antimicrobial activities of N-laurylpyridinium iodides (19 compounds) having various substituents (methyl, ethyl, propyl, amino, carboxyl, and carbomoyl groups) on the pyridine nuclei were studied against *Escherichia coli* K12, *Bacillus subtilis var. niger*, *Aspergillus niger*, and *Candida utilis* with respect to their chemical structures. The N-lauryl-2,4,6-trimethylpyridinium iodide had the highest antimicrobial activity, whereas N-lauryl-2-carboxylpyridinium iodide had the lowest activity.

In general it can be seen from Table 13–12 that electron-releasing groups on the pyridine nucleus such as amino or methyl group markedly enhanced the activities, and the electron-attracting groups such as carboxyl or carbamoyl group highly reduced them. There was a clearly linear relationship between the antimicrobial activities and the acidic dissociation constant (pK_a) for the corresponding pyridines, and the same relationships exist between the pK_a and both bacteriolytic and bactericidal activities.

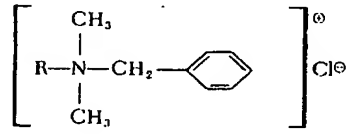
In conclusion the findings obtained suggest that the antimicrobial activities of the N-laurylpyridinium iodides are linearly dependent on the electron density of the ammonium moiety. It needs to be mentioned here, however, that the authors did not report on the stability of the iodide salts. It is well known that iodides under acidic conditions in the presence of oxygen and light can be oxidized to iodine, which is a well known biocide (Merianos, 1988). The only commercially used pyridinium quaternary is cetylpyridinium chloride, as the active ingredient in mouthwash (Cepacol), and in preservatives in pharmaceutical preparations. Economic considerations have prevented the commercialization of the substituted N-laurylpyridinium iodide quaternaries.

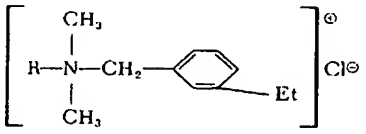
Kourai and coworkers (1989) reported on the relationship between hydrophobicity of bacterial cell surface and drug susceptibility to alkylpyridinium iodides. The value of antimicrobial activity (log 1/MIC) of quaternary iodides having long alkyl chains (hexyl, octyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl) against each bacterium was regarded as the drug susceptibility. The partition coefficient of bacterial cells between n-hexadecane and physiologic saline was regarded as representing their hydrophobicity of the cell surface.

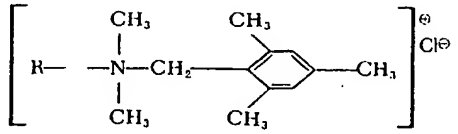
The logarithm of the coefficient was employed as a measure of the hydrophobicity at 37°C. The hydrophobicity of the gram-negative bacteria was always higher than that of the gram-positive bacteria. The susceptibility of the bacteria to each of the alkylpyridinium iodides was analyzed by use of the hydrophobicity, and a quantitative relationship between the susceptibility and their hydrophobicity was found. These findings eventually lead to a conclusion that the first step of the antimicrobial action of the iodides is a hydrophobic interaction between the cell surface and the iodide, and that the magnitude of the drug-susceptibility of the bacteria depends upon the hydrophobicity of the cell surface. Daoud et al. (1983) investigated the relationships between physicochemical properties and the antimicrobial activity of a homologous series of alkyldimethylbenzyl ammonium chlorides

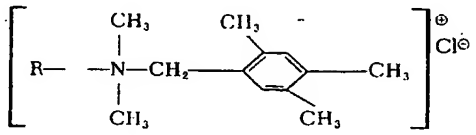
Table 13–10. *AOAC Germicidal and Detergent Sanitizers Test Method* Escherichia Coli *ATCC No. 11229 200 ppm Active Quat to Give 99.999% Reduction of* E. Coli *in 30 Seconds*

	Hard Water Tolerance (ppm) $CaCO_3$			
Alkyl	*A*	*B*	*C*	*D*
R_{12}	100	400	900	1250
R_{14}	700	700	1000	700
R_{16}	400	400	600	500
R_{18}	100	300	550	350
$R_{12-(70)\ 14\ (30)}$	650	1000	1300	1200

(A) 

(B) 

(C) 

(D) 

U.S. Patents 3,525,793, 3,472,939

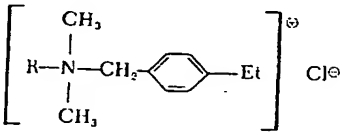
against a variety of microorganisms, and considered whether benzalkonium chloride mixtures could be improved as antimicrobial agents through a more rational choice of alkyl chain length composition. The minimum growth inhibitory concentrations (MIC) of various members of the homologous series of alkyldimethylbenzyl ammonium chlorides were determined against 12 strains of microorganisms, representative of gram-positive and gram-negative bacteria, yeast, and fungi.

The critical micelle concentrations and octanol-water partition coefficient (P) were measured for alkyldimethylbenzyl ammonium chlorides. The partition coefficients were calculated as the ratio of the concentration in the aqueous and oil (alcohol) phases expressed as the means of the replicate determination. Physicochemical data such as log P and log 1/MIC were subjected to multiple regression analysis to produce quadratic equations of the general form:

$$\text{Log } 1/\text{MIC} = a + b \log P + C [\log P]^2$$

The log P values were plotted against biologic activity for a series of compounds, and a parabolic relationship was obtained. Log P_o would in this instance be the value of log P for compounds with optimal biologic activity. This value of log P_o will vary between different types of organisms. Many such parabolic relationships have been demonstrated, including several for quaternary ammonium compounds (Hansch and Clayton, 1973; Hansch and Fujita, 1964). The levels of antimicrobial activity were observed to be parabolically related to the alkyl chain length, and thereby to log P. The chain lengths with optimal activity varied from organism to organism, reflecting differences in their cell-wall structures. The lower chain lengths [C_{10} to C_{12}] were more active against yeast and fungi, whereas gram-negative organisms were most susceptible towards the more lipophilic C_{16} compounds. This was probably a consequence of the lipophilic nature of the gram-negative cell wall and of the difficulties often encountered by hydrophilic molecules to traversing it. As result gram-negative microorganisms were the least sensitive towards all of the homologues, MIC values being approximately 10 times greater than those towards the gram-positive organisms and the fungi. The possibility of dual binding sites has experimental support from the observations of Salt and Wiseman (1968) working with cetyltrimethyl ammonium bromide, which binds in two stages, representing high- and low-affinity binding sites for the drug. Such a mechanism implies potential synergy in combination of benzalkonium chlorides and might influence the choice of alkyl chain components for preservative mixture. Hansch and Fujita (1964) and Lien et al. (1968) reported that the differences in the biologic activity of chemically related antimicrobials, acting at similar sites within the cell, would reflect their relative ease of penetration through the various liquid barriers of the cells and their ability to react at that site. The outer components of the cells were considered as a series of aqueous and lipophilic layers. Sub-

Table 13–11. *AOAC Germicidal and Detergent Sanitizers Test* Escherichia Coli *ATCC No. 11229 200 PPM Active Quat to Give 99.999% Reduction of* E. Coli *in 30 Seconds*



U.S. Patent 3,525,793

R		Hard Water Tolerance PPM	
C_{12}	C_{14}	Calculated	Determined
100	0	400	400
95	5	415	500
90	10	430	750
85	15	445	1000
82.5	17.5	453	1050
80	20	460	1110
77.5	22.5	468	1000
75	25	475	1050
70	30	490	1000
65	35	505	1000
60	40	520	900
55	45	535	1000
50	50	550	900
45	55	565	1000
40	60	580	900
35	65	595	800
30	70	610	750
25	75	625	800
20	80	640	800
10	90	670	800
5	95	685	600
0	100	700	700

stances of low water solubility would be unable to penetrate these aqueous layers and would accumulate within the lipid regions; similarly, those with low oil solubility would be unable to cross the lipophilic barriers. Compounds between these two extremes must exist that possess the optimum balance between hydrophilicity and lipophilicity for traversing the cell barriers.

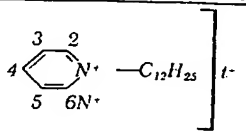
In summary, antimicrobial activity of the compounds was found to be a parabolic function of their lipophilicity and maximized with n-alkyl chain length of between C_{12} and C_{16}. Generally yeast and fungi were most sensitive towards C_{12}, gram-positive bacteria towards C_{14}, and gram-negative bacteria towards C_{16}. The gram-negative cells were the most resistant towards all the compounds, and gram-positive cells the least.

Tomlinson and coworkers in 1977 reported on the effect of colloidal association on the measured activity of alkyldimethyl benzylammonium chlorides (ADBAC) homologous series C_8 to C_{18} against *Pseudomonas aeruginosa* (ATCC 9027). The critical micelle concentrations (CMC) is an important factor in the study of antimicrobial activity of cationics; at high concentrations of ADBAC, formation of a micellar state of the salt is interfering with the cationics' antibacterial action. Tomlinson et al. (1977) questioned the normally accepted parabolic relationship reported to exist between the lipophilicity of many quaternary ammonium salts and their antibacterial activities. The high concentration of nutrient salts, which is used in screening, would alter the concentration of monomeric quaternary ammonium salt. This may be responsible for some of the parabolic relationships, rather than the intrinsic antibacterial activity of the compounds. As early as 1953 it was reported that for the homologous series of cationic surface active agents, a parabolic relationship could exist between their antibacterial properties and their hydrophobic character. Thus, there is a linear relationship between activity and alkyl chain length with increased carbon number up to a maximum of between C_{12} and C_{14}, at which region there is observed a decrease in activity. In Table 13–3, the work of Cutler et al. (1967) showed that with broth dilution tests a peak activity against various microorganisms could be observed at an alkyl chain length of 14 carbon atoms. The turndown in activity is probably related to more than one physical property of the compounds: (a) The longer the chain the greater the tendency for the molecule to be adsorbed at the surface of a bacterium; (b) There is a reduction of aqueous solubility of the molecule as the carbon number is increased. These workers failed to discuss the relevance of micellization to the thermodynamic state of the ADBAC molecule in the test system. Salt additives are known to increase the tendency of ionic surface active agents to micellize in water. In some instances the micellar molecular weights can increase, indicating a change in micellar type.

In conclusion, Tomlinson et al. (1977) have measured the antibacterial activities of ADBAC homologous by the minimum inhibitory concentration procedure and by a sterilization kinetics test carried out in deionized water. There was a log-linear relationship between activity measured by kinetics test and carbon number. With the MIC method there was a log-linear relationship up to C_{14}, then there was a turndown in activity. So consideration of the colloidal association of ADBAC in deionized water and in a simple salts growth media led these authors to suggest that use of high concentrations of nutrient salts in MIC tests will lower the effective concentration of the surface active agents. This change may be responsible for the turndown in activity observed in MIC tests, and that in such circumstances the MIC test does not give a true reflection of the intrinsic activity of the compounds.

Jono and coworkers (1986) investigated the effect of alkyl chain length of benzalkonium chloride (BAC) on the bactericidal activity and binding to organic materials during a short contact time (10 s, 30 s, 1 min, 10 min) by counting survivors. Table 13–13 lists the results. The effect of human serum and dried yeast on the bactericidal activity of ADBAC standard blend OSN Alkyl[C_{12}—59

Table 13–12. *Minimum Inhibitory Concentration of N-Laurylpyridinium Iodides Against* Escherichia Coli *K12*, Bacillus Subtilis *Var.* Niger, Candida Utilis, *and* Aspergillus Niger



No.	2	3	4	5	6	Abbreviation	MICs(M)* E. coli K12	B. subtilis var. niger	C. utilis	A. niger
1.	H	H	H	H	H	Py	53	5.6	61	85
2.	CH_3	H	H	H	H	2-Me-Py	21	3.6	26	26
3.	H	CH_3	H	H	H	3-Me-Py	51	4.9	59	82
4.	H	H	CH_3	H	H	4-Me-Py	31	4.6	45	72
5.	CH_3	H	CH_3	H	H	2,4-Me-Py	20	4.0	32	35
6.	CH_3	H	H	H	CH_3	2,6-Me-Py	20	5.0	30	40
7.	H	CH_3	CH_3	H	H	3,4-Me-Py	40	2.7	20	35
8.	H	CH_3	H	CH_3	H	3,5-Me-Py	74	5.5	17	69
9.	CH_3	H	CH_3	H	CH_3	2,4,6-Me-Py	2.6	0.39	0.49	29
10.	H	$CONH_2$	H	H	H	3-$CONH_2$-Py	71	20	210	67
11.	H	H	$CONH_2$	H	H	4-$CONH_2$-Py	84	24	220	77
12.	COOH	H	H	H	H	2-COOH-Py	1100	380	640	460
13.	H	COOH	H	H	H	3-COOH-Py	780	280	430	400
14.	H	H	COOH	H	H	4-COOH-Py	1000	350	700	460
15.	NH_2	H	H	H	H	2-NH_2-PY	44	10	13	23
16.	H	NH_2	H	H	H	3-NH_2-PY	29	1.8	3.8	35
17.	H	H	NH_2	H	H	4-NH_2-PY	10	1.6	7.0	23
18.	C_2H_5	H	H	H	H	2-C_2H_5-Py	45	12	160	130
19.	C_3H_7	H	H	H	H	2-C_3H_7-Py	24	6.3	53	120

*MICs against bacteria and yeast were measured by a Hitachi Recording Incubator, and MICs against mold by the test-tube dilution method.

to 63%, C_{14}—29 to 34%, C_{16}—6.8 to 7.2%]dimethylbenzyl ammonium chloride and pure C_{12}BAC, C_{14}BAC, C_{16}BAC was compared against 13 strains of bacteria.

It was concluded that from the practical point of view C_{12} benzalkonium chloride was the most effective component of the homologues series in the presence of organic materials. The bactericidal activity of BAC was inhibited by both dried yeast and human serum. The inhibition by 2.5% dried yeast was stronger than that by 10% human serum. As the carbon chain was increased, the inhibition increased. The C_{12}BAC was bactericidal against *Pseudomonas cepacia*, *Pseudomonas aeruginosa*, *Achromobacter guttatis*, *Alcaligenes faecalis*, and *Serratia marcescens* at 5000 ppm in a suspension of 2.5% dried yeast and at 2500 ppm in a 10% solution human serum at 1 min of contact, but C_{14}BAC and C_{16}BAC could not kill them at 10,000 ppm at 10 min. Many factors might be affected by the alkyl carbon length of BAC, producing changes in the bactericidal activity; for example, (1) aqueous solubility; (2) aqueous critical micelle concentration; (3) lipophilicity; and (4) the cell surface characteristics of the microorganisms used. The C_{12}BAC and C_{14}BAC were effectively bactericidal with all bacteria tested, even at a short contact time (30 s to 1 min). The inhibition of bactericidal activity by organic materials was least for the C_{12}BAC (Table 13–13). To clarify the relationship between inhibition of bactericidal activity of BAC by organic materials and binding of BAC to them, the authors assayed unbound C_{12}BAC and C_{14}BAC in a solution of bovine serum albumin by HPLC. As the carbon chain was lengthened, aqueous solubility decreased.

When the carbon chain is longer than C_{14}, the solubility and the critical micelle concentration of BAC are extremely low. The binding to bovine serum albumin is increased with increase in the carbon chain; the C_{14}BAC bound to bovine serum albumin 2.5 to 3.7 times more than C_{12}BAC did. The bactericidal activity of BAC in a solution of bovine serum albumin depended on the amount of unbound BAC. The results in Table 13–13 show that other factors in human serum also inhibited the activity. Human serum tended to stimulate bactericidal activity against *Staphylococcus aureus* and *Pseudomonas cepacia* when the contact time was short. Human serum may make the surface of some bacteria more sensitive to BAC. These results suggest that C_{12}BAC is the most effective component of the homologous series of BAC in the presence of organic materials. The greater the content of C_{12}BAC in the blends, the more effective the mixture will be as a sanitizer from the practical point of view.

ANTIMICROBIAL ACTIVITY

The assessment of the antimicrobial activity of the quaternary ammonium compounds has, in some recent instances, demonstrated a lack of scientific objectivity. Those statements about antimicrobial quaternary ammonium compound testing that, by the selection of the

Table 13–13. *Effect of Human Serum and Dried Yeast on Bactericidal Activity of Homologues of the Long-Chain Alkyl Benzalkonium Chlorides*

Organism		Bactericidal Concentration ($\times 10^1$ μg/ml)[a]															
		OSN				C_{12}-BAC				C_{14}-BAC				C_{16}-BAC			
		10s	30s	1 min	10 min	10s	30s	1 min	10 min	10s	30s	1 min	10 min	10s	30s	1 min	10 min
S. aureus FDA 209P	DW	0.5	0.5	0.2	0.05	5	1	0.5	0.1	1	0.4	0.1	0.025	0.4	0.2	0.025	0.01
	HS	1	0.5	0.5	0.5	1	1	0.5	0.5	5	1	0.5	0.5	0.5	0.5	0.5	0.5
	DY	5	5	2.5	2.5	5	5	2.5	2.5	5	5	2.5	2.5	>10	2.5	2.5	2.5
S. epidermidis IFO 3762	DW	0.2	0.1	0.0125	0.006	1	0.4	0.4	0.025	0.8	0.025	0.025	0.006	0.05	0.006	< 0.006	< 0.006
	HS	0.5	0.5	0.5	0.5	2.5	1	0.5	0.5	0.5	0.5	0.5	0.5	2.5	0.5	0.5	0.5
	DY	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	1
P. cepacia ATCC 17774	DW	5	1	1	0.4	1	0.8	0.5	0.5	>10	0.5	0.2	0.1	>10	>10	0.2	0.05
	HS	2.5	0.5	0.5	0.5	2.5	1	0.5	0.5	>10	5	2.5	0.5	>10	>10	>10	2.5
	DY	10	5	5	2.5	5	5	5	2.5	>10	>10	10	5	>10	>10	>10	>10
P. cepacia H130	DW	5	5	0.2	0.1	5	5	1	0.5	10	5	5	0.2	>10	>10	>10	2.5
	HS	5	1	0.5	0.5	5	1	1	0.5	5	5	2.5	1	>10	>10	>10	5
	DY	>10	5	5	2.5	10	5	5	5	>10	>10	>10	10.5	>10	>10	>10	>10
P. aeruginosa IFO 13736	DW	0.05	0.05	0.025	0.01	0.1	0.1	0.05	0.025	0.05	0.5	0.025	0.001	0.1	0.025	0.025	0.01
	HS	2.5	1	0.5	0.5	10	5	0.5	0.5	10	5	2.5	1	>10	>10	5	1
	DY	10	10	5	5	10	5	5	5	>10	>10	10	5	>10	>10	10	5
P. aeruginosa 82-2-32	DW	0.1	0.1	0.025	0.01	0.2	0.1	0.1	0.025	0.2	0.2	0.025	0.01	10	0.1	0.05	0.01
	HS	2.5	2.5	0.5	0.5	5	5	2.5	1	10	5	2.5	1	5	5	0.5	0.5
	DY	>10	10	10	10	10	5	5	5	>10	>10	10	10	>10	>10	>10	>10
P. mirabilis ATCC 21100	DW	0.2	0.2	0.05	0.05	0.4	0.2	0.01	0.01	0.2	0.05	0.05	0.025	0.1	0.05	0.01	0.01
	HS	5	2.5	0.5	0.5	5	1	1	0.5	10	2.5	2.5	1	>10	5	5	1
	DY	>10	5	5	2.5	10	5	5	2.5	>10	10	5	5	>10	>10	>10	10
P. mirabilis 82-1-4	DW	5	0.2	0.1	0.01	1	0.4	0.2	0.05	1	0.1	0.05	0.05	>10	0.2	0.05	0.025
	HS	5	1	0.5	0.5	5	2.5	1	1	10	2.5	2.5	0.1	>10	>10	2.5	0.5
	DY	>10	5	5	5	10	10	5	5	>10	>10	5	5	>10	>10	>10	10
P. morganii 82-2-11	DW	0.5	0.2	0.1	0.05	0.2	0.2	0.05	0.05	1	0.05	0.01	0.01	1	0.05	0.05	0.025
	HS	2.5	1	0.5	0.5	10	5	0.5	0.5	10	5	1	1	>10	>10	5	1
	DY	5	5	5	5	10	5	5	5	10	10	5	5	>10	>10	>10	10
S. marcescens 82-2-52	DW	5	0.4	0.2	0.05	>10	0.4	0.4	0.025	>10	0.4	0.2	0.025	>10	>10	0.025	0.025
	HS	5	2.5	1	0.5	10	5	1	0.5	>10	>10	5	2.5	>10	>10	>10	2.5
	DY	>10	>10	5	5	>10	5	5	5	>10	>10	10	5	>10	>10	>10	10
Flavobacterium sp. 82-1-98	DW	0.1	0.05	0.05	0.025	0.2	0.05	0.05	0.025	0.2	0.05	0.025	0.025	0.2	0.1	0.025	0.01
	HS	2.5	0.5	0.5	0.5	5	1	0.5	0.5	5	5	1	0.5	10	5	2.5	0.1
	DY	2.5	2.5	2.5	2.5	5	2.5	2.5	1	10	10	5	1	10	5	5	2.5
A. guttatis A-39	DW	5	5	1	0.2	>10	>10	1	1	>10	>10	10	1	>10	>10	>10	0.5
	HS	5	5	1	0.5	5	5	1	1	>10	>10	>10	>10	>10	>10	>10	>10
	DY	>10	>10	>10	2.5	>10	>10	5	5	>10	>10	>10	>10	>10	>10	>10	0.2
A. faecalis 572	DW	1	1	0.2	0.1	1	0.4	0.2	0.2	>10	0.2	0.2	0.2	>10	>10	>10	10
	HS	10	2.5	1	0.5	10	5	2.5	0.5	>10	10	10	1	>10	>10	>10	>10
	DY	>10	>10	10	5	>10	10	5	5	>10	>10	>10	5	>10	>10	>10	>10

[a]Killed from 10^6 to <10 cfu/ml at 25 C. DW, deionized water; HS, 10% human serum; DY, 2.5% dried yeast. OSN, alkyl(59–63% $C_{12}H_{25}$–, 29–34% $C_{14}H_{29}$–, 6.8–7.2% $C_{16}H_{33}$–) dimethylbenzylammonium chloride.

Joyo, K., et al. 1986. Chem. Pharm. Bull., *34*, 4215–4224.

proper test method, the compounds may appear as good disinfectants (Mallman et al., 1946), or that the phenol coefficient test cannot be used to test quaternary ammonium compounds (Sykes, 1965), or that disinfection end points obtained via inoculated carrier tests require much larger amounts of compound over those observed from phenol coefficient testing (Mallman and Hanes, 1945; Stuart et al., 1953), or that tests with quaternary ammonium compounds measure bacteriostatic activity instead of bactericidal activity (Klarmann and Wright, 1946) are grossly misleading unless some additional interpretive and qualifying information is provided. Unfortunately, a large amount of the experimental work of the middle 1930s, immediately following Domagk's publication (1935), did indeed indicate a widespread spectrum of antimicrobial activity, including sporicidal and tuberculocidal activity at extremely low concentrations of quaternary ammonium compounds. There is little doubt that this was a result of inadequate neutralization of the test material carried over to the subculture medium, so that a static condition produced in the subculture was mistaken for destructive activity.

However, it must be remembered that the entire area of neutralization to prevent carryover stasis was inadequately dealt with at the time. A firm distinction between the biostatic and biocidal activities of an antimicrobial was not always determined. The necessity to obviate stasis from the subculture medium was not always demanded as a requisite of antimicrobial testing, and the neutralization with specific chemical moieties used for individual classes of antimicrobials was not always insisted upon. Any neutralization that occurred utilized dilution of the carryover antimicrobial from the aliquot sample to the volume of subculture medium to obtain a less than static concentration of the antimicrobial in the subculture medium.

More recently, the successful identification of discrete chemicals as specific quaternary ammonium compound neutralizers, which may be incorporated into subculture media or into diluents, has made it mandatory that antimicrobial testing be performed only in the presence of such neutralizers (Quisno et al., 1946). Consequently, the more recent experimental results with quaternary ammonium compounds cannot be discredited as static instead of destructive. The statement that the quaternary ammonium compounds may be made to look good by the proper test selection (Mallman et al., 1946) may be applied to any antimicrobial chemical.

Certainly, a manipulation of the various test parameters such as type and amount of soil, selection of test organisms, and many others will bias the results, so that any chemical product will appear as a good or poor antimicrobial agent. Of the many inadequacies attributed to the phenol coefficient test method (Klarmann and Wright, 1946; McCulloch, 1947), the most pertinent objection was its use as an index of actual disinfection levels for quaternary ammonium compounds (Sykes, 1965). However, this same objection may be raised for the entire spectrum of modern disinfectant products, because extrapolation from the phenol coefficient to recommended use-dilution for a product by way of some arbitrary factor is invalid and untenable in any and every instance (Klarmann, 1959; Ortenzio et al., 1961). If the in-use characterization of a disinfectant is divorced from the phenol coefficient test, the method becomes entirely useful to describe the antimicrobial activity of a substance as the concentration that will destroy a bacterial suspension in 5-, 10-, or 15-minute contact periods. The method, which fixes many independent variables such as operation equipment, media, temperature, and volumes of test solution and inoculum, may be applied with some minor reservations to any species of microorganism. That carrier type test methods provide lower dilution end points than the phenol coefficient method has already been stated for quaternary ammonium products (Mallman and Hanes, 1945; Stuart et al., 1953) and, more importantly, for other kinds of chemical products (Klarmann, 1959; Ortenzio et al., 1961). The dynamics of testing carriers prepared with inocula as dried films of microorganisms provide a more severe test than the liquid inoculum system of the phenol coefficient method. This feature of carrier-type tests has led to the development and use of these tests by the United States regulatory agencies to measure bactericidal, sporicidal, tuberculocidal, and sanitizing activity (Official Methods, AOAC, 1984).

Of special note are the efforts to determine the validity and accuracy of product-label statements and claims made by manufacturers of disinfectant products for sale in the United States. This regulation of antimicrobials intended for treatment of inanimate surfaces was formerly exercised by the United States Department of Agriculture and currently by the United States Environmental Protection Agency. It includes deliberate scrutiny of a product for antimicrobial efficacy and toxicologic and chemical properties in compliance with the product-label claims and other attendant information—all prior to the initial sale of the product. Further, the agency is required to monitor future production obtained on a periodic inspection schedule. To facilitate the antimicrobial examination, a number of testing protocols are prescribed to examine specific antimicrobial activity (Greene and Petrocci, 1980).

For those catalogued as AOAC methods, the reader is referred to the AOAC publications (Official Methods of Analysis of the AOAC, 1984) for the purpose, scope, precise methods, and application areas served by these tests. In addition to the AOAC methods, the Environmental Protection Agency recommends methods to determine the sanitizing activity on inanimate, nonfood contact surfaces, the initial and residual bactericidal and bacteriostatic activity of treated laundry fabric, the sanitizing activity on carpeting, the virucidal activity on environmental surfaces, and the initial and residual fungicidal and fungistatic activity on inanimate surfaces. This requirement to test products by specified protocols pro-

vides a useful means to collect data that may be compared, and so to more accurately characterize the antimicrobial activity of a substance. The recent installations of prescribed test methods by other agencies and in other countries is a most salutary development to eliminate the confusing conclusions developed heretofore from many different kinds of experimental studies.

Quaternary ammonium compounds have been tested by a multitude of different procedures, which may be generally classified as (1) In vitro static or minimum inhibitory level tests, usually as broth dilution tests; (2) In vitro or killing dilution tests, with phenol coefficient as an example; (3) Simulated use-dilution tests, including inoculated carriers of representative surfaces such as stainless steel, unglazed porcelain, and fabric; and (4) In-use tests, which use the product in the actual environment (Greene and Petrocci, 1980). The results of such testing with quaternary ammonium compounds may be summarized as follows: the active quaternary ammonium compounds sold as items of commerce are algistatic, bacteriostatic, tuberculostatic, sporostatic, and fungistatic at low concentration levels of 0.5 to 5 ppm (Hueck et al., 1966; Freelander, 1940; Schneider, 1935); they are algicidal, bactericidal, fungicidal, and virucidal against lipophilic viruses at medium concentration levels of 10 to 50 ppm (Lawrence, 1950; Petrocci et al., 1974; Klein and Deforest, 1963); they are not tuberculocidal or sporicidal or virucidal against hydrophilic viruses at high concentration levels (Klein and Deforest, 1963; Smith et al., 1950; Davies, 1949). They are bactericidal to both gram-positive and gram-negative bacteria, with some evidence for greater activity against the gram-positive bacteria (Quisno and Foter, 1946). In this regard, it should be remembered that the gram-negative bacteria as a group, and especially the pseudomonas species, are more resistant to all the antimicrobial compounds currently available.

The quaternary ammonium compounds are bactericidal agents in acid and alkaline environments, with some evidence for greater activity in the alkaline range (Gershenfeld and Perlstein, 1941). The following descriptions of antimicrobial results are presented as representative data for quaternary ammonium compounds. They are compiled and prepared from the published scientific literature, from Onyx (Stepan) unpublished work, from brochures and data sheets supplied by the quaternary ammonium compound manufacturers, and from patents issued for quaternary ammonium compounds. It is important to recognize the absence of bias in information supplied by the manufacturers' literature that is carefully scrutinized by the regulatory agencies in advance of the sale of the chemical. The bacteriostatic, fungistatic, and algistatic activities of fatty nitrogen compounds, including quaternary ammonium compounds, were described

Table 13–14. *Inhibiting Concentrations (in PPM) of Fatty Nitrogen Compounds For Some Bacteria, Fungi, and Algae*

	Bacteria			
	G–N		*G–P*	
Compound	*Escherichia coli*	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*
Benzethonium chloride	1,000	300	3	3
Benzalkonium chloride	200	300	3	4
Dodecyltrimethyl ammonium chloride	500	500	5	5
Dodocylbenzyldimethyl ammonium chloride	750	750	2	2
Cocobenzyldimethyl ammonium chloride	225	225	2	2
Didecyldimethyl ammonium chloride	225	750	0.7	7

	Fungi			
Compound	*Aspergillus niger*	*C. globosum*	*M. verrucaria*	*T. viridae*
Benzethonium chloride	300	30	300	200
Benzalkonium chloride	60	10	40	80
Dodecyltrimethyl ammonium chloride	500	50	500	500
Dodocylbenzyldimethyl ammonium chloride	75	7	150	75
Cocobenzyldimethyl ammonium chloride	20	7	20	20
Didecyldimethyl ammonium chloride	75	7	20	20

	Algae			
Compound	*Ch. vulgaris*	*Stigeoclonium species*	*A. cylindrica*	*Os. tenuis*
Benzethonium chloride	3	1	1	1
Benzalkonium chloride	1	0.7	1	0.6
Dodecyltrimethyl ammonium chloride	50	5	5	0.5
Dodocylbenzyldimethyl ammonium chloride	0.2	0.2	0.2	0.2
Cocobenzyldimethyl ammonium chloride	2	0.5	2	0.7
Didecyldimethyl ammonium chloride	2	0.7	0.2	0.7

by Hueck et al. (1966); data selected therefrom for the quaternary ammonium compounds are presented in Table 13–14. The killing dilutions for some chemically different, commercially available quaternary ammonium compounds after a 10-minute contact period at 20°C by the phenol coefficient method against the recommended test organisms *Staphylococcus aureus* and *Salmonella typhosa* are compiled from the manufacturers' brochures (e.g., Brochure: Hyamine, Brochure: Roccal, BTC 2125M BTC, Brochure: Bardac) as Table 13–15.

These are at high dilutions against both organisms, and they are at significantly different dilutions depending on the specific chemical structure of the quaternary compound. The effect of the size of the quaternary molecule on the antimicrobial activity of a homologous series of alkyldimethylbenzyl ammonium chlorides is well documented by Cutler et al. (1966). They demonstrated a maximum activity as the highest 10-minute killing dilution against *Staphylococcus aureus* and *Salmonella typhosa* and *Pseudomonas aeruginosa* for the C_{14} member compound tested. Their results are shown in Table 13–3. Additional evidence for the structure and antimicrobial activity relationship is offered by the work of Petrocci et al. (1974), who evaluated the interference of hard water with the sanitizing activity against *Escherichia coli* of a group of dialkyldimethyl ammonium chloride compounds of varying long-chain lengths. This comparison of the hard-water tolerances, which are defined as the maximum concentrations of synthetic hard water (as $CaCO_3$), in which 200 ppm of active quaternary compound will continue to effectively sanitize (at 99.999% reduction) a suspension of *Escherichia coli* after a 30-second contact period, are displayed in Table 13–16.

Quisno's work with cetyl pyridinium chloride (Quisno and Foter, 1946) illustrates several characteristics of quaternary ammonium compounds, such as the greater activity for gram-positive bacteria over gram-negative bacteria (Table 13–17), and the positive effect of temperature and the negative effect of organic matter on antimicrobial activity (Table 13–18).

Lawrence (1950) described the effect of pH on the antibacterial activity of an alkyldimethylbenzyl ammonium chloride at 5-, 10-, and 15-minute contact periods against *Staphylococcus aureus*. A portion of these data is available as Table 13–19. Over many years, Petrocci has examined the fungicidal activity of many quaternary structures against *T. interdigitale* (Petrocci, unpublished). Some of these data are available as Table 13–20. This small section of antimicrobial activity is only a minute portion of the total information that has been published and is available for examination. The space limitations of this chapter do not permit a complete listing of this information; instead, I have attempted to select and present representative data that are compatible with the bulk of current opinions in the field.

TOXICOLOGY

On March 4, 1987, the EPA issued a Data Call-In Notice requiring all registrants of antimicrobial active ingredients to submit subchronic and chronic toxicologic data to support the continued registration of their products. The EPA will permit registrants to select among several options for ways to comply with the data requirements of this notice. The EPA has concluded that it should be possible to evaluate risk by acquiring exposure data and by acquiring toxicity data under a tiered approach. The three-tier studies may be summarized as follows:

A. *The first-tier studies:*
   1. 90-days dermal
   2. 90-days inhalation
   3. Teratogenicity 1st
   4. Mutagenicity

B. *The second-tier studies:*
   1. Subchronic feeding

Table 13–15. *Killing Dilutions of Commercially Available Quaternary Ammonium Germicides for 10-Minute Contact Periods at 20°C from Phenol Coefficients in Manufacturers' Brochures*

Chemical	*Killing Dilutions vs.*	
	Staphylococcus aureus	*Salmonella typhosa*
Di-isobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride	1/25,000	1/25,000
40% Methyldodecylbenzyl trimethyl ammonium chloride, 10% methyl-dodecylxylylene bis (trimethyl ammonium chloride)	1/24,000	1/22,500
N-alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethyl benzyl ammonium chlorides	1/45,000	1/45,000
Benzyldimethyltetradecyl ammonium chloride	1/42,800	1/67,500
N-alkyl (60%C_{15}, 30%C_{16}, 5%C_{12}, 5%C_{18}) dimethylbenzyl ammonium chlorides	1/44,000	1/39,600
25% *N*-alkyl (68%C_{12}, 32%C_{14}) dimethylethylbenzyl ammonium chlorides	1/55,500	1/54,300
N-alkyl (98%C_{17}, 20%C_{14}) dimethyl 1-naphthylmethyl ammonium chlorides	1/60,000	1/58,500
N-alkyl (50%C_{12}, 30%C_{14}, 17%C_{16}, 3%C_{18}) isoquinolinium bromides	1/38,000	1/40,000
Octyldodecyldimethyl ammonium chlorides	1/61,800	1/57,500
Alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethylbenzyl ammonium saccharinates	1/43,500	1/40,500
Didecyldimethyl ammonium chlorides	1/63,000	1/84,000

Table 13–16. *Hard Water Tolerances (PPM $CaCO_3$) of Dialkyldimethyl Quaternary Ammonium Compounds with Long-Carbon Chains (R_1 + R_2) that Total 19 to 22 Carbons*

Totals Carbons (R_1 + R_2)*	Hard Water Tolerance (ppm $CaCO_3$)						
19	700	800	500	550			
20	500	900	1200	1400	1100		
21	300	700	900	1300	1300		
22	400	550	750	900	800	650	
	6	7	8	9	10	11	R_1 carbon length

*R_2 carbon chain length obtained by subtracting R_1 carbon chain length from the R_1 + R_2 total carbon chain length.

Table 13–17. *Germicidal Activity of Cetyl Pyridinium Chloride Aqueous Solution*

Organism	No. Strains Tested	Average Critical Killing Dilution In Terms of Active Ingredients at 37°C
		No serum
Staphylococcus aureus	5	1:83,000
Staphylococcus albus	1	1:73,000
Streptococcus viridans	1	1:42,500
Streptococcus hemolyticus	2	1:127,500
Neisseria catarrhalis	2	1:84,000
Diplococcus pneumoniae I	1	1:95,000
Pseudomonas aeruginosa	2	1:5,800
Klebsiella pneumoniae	2	1:49,000
Corynebacterium diphtheriae	1	1:64,000
Mycobacterium phlei	1	1:1,500
Eberthella typhosa	5	1:48,000
Escherichia coli	2	1:66,000
Proteus vulgaris	2	1:34,000
Shigella dysenteriae	1	1:60,000
Shigella paradysenteriae (Flexner)	2	1:52,000
Shigella paradysenteriae (Hiss)	1	1:49,000
Shigella sonne	2	1:68,000

2. Teratogenicity 2nd
3. Dermal absorption
4. 90-days neurotoxicity

C. *The third-tier studies:*
1. Chronic feeding, two species
2. Oncogenicity, two species
3. Reproduction
4. Metabolism to clarify issues concerning structure activity relationships

On February 26, 1988, the EPA clustered the quaternary ammonium compounds into four groups, so that the toxicity study would be facilitated by selecting one member from each group for testing.

Group I: The straight-chain alkyl or hydroxyalkyl quaternaries; the most common product in this group is the twin chain quats. The EPA will allow a full set of subchronic and chronic toxicity studies on didecyldimethyl ammonium chloride and teratology studies on alkyltrimethyl ammonium chloride.

Group II: The nonhalogenated substituted benzyl and alkyldimethylbenzyl ammonium compounds (ADBAC); namely benzalkonium chlorides. The EPA will allow a full set of subchronic and chronic studies on alkyl[C_{12}—40%, C_{14}—50%, C_{16}—10%] dimethylbenzyl ammonium chloride. The EPA notes that after evaluating the data it expects to receive on the Group I and II compounds, it may also require one or more studies on alkyl[C_{12}—68%, C_{14}—32%]dimethylethylbenzyl ammonium chloride, to determine whether the Group I and ADBAC data is representative of the toxicity of the ethylbenzyl quats.

Group III: The alkyl[di- and tri- chlorobenzyl]dimethyl ammonium compounds. The EPA will allow a full set of subchronic and chronic studies on alkyl[C_{12}—5%, C_{14}—90%, C_{16}—5%] dimethyl 3,4-dichlorobenzyl ammonium chloride.

Group IV: The heterocyclic ammonium compounds with unusual substituents. In this group the EPA was unable to reach a conclusion in testing a small subset of quats. Each chemical in this category must be tested separately, unless registrants can develop a testing scheme using representative compounds that is acceptable to the EPA. Representative compounds in *Group IV* are (a) alkyl[C_{12}/C_{16}] substituted Picolinium compounds; (b) alkyl[C_8/C_{18}] imidazolinium compounds; (c) alkyl[C_{14}/C_{18}] N-ethyl-morpholinium compounds; and (d)

Table 13–18. *Effect of Temperature and Serum Upon Germicidal Activity of Cetyl Pyridinium Chloride*

Organisms	Critical Killing Dilutions Expressed in Terms of Active Ingredients			
	37°C		20°C	
	No Serum	10% Bovine Serum	No Serum	10% Bovine Serum
Staphylococcus aureus	1:110,000	1:11,500	1:67,500	1:6,750
Eberthella typhosa	1:83,500	1:4,000	1:62,500	1:1,300

Table 13–19. *Effects of pH on the Antiseptic Efficiency of a Quaternary Ammonium Germicide Against* Staphylococcus Aureus

Quaternary Ammonium Compound Conc.	*Acid or Alkali per 100 ml*	*pH*	*Bacterial Growth after Minutes of Exposure*		
			5	*10*	*15*
1:15,000	0.10 ml N/10 HCl	4.3	–	–	–
1:15,000	0.25 ml N/10 HCl	3.8	–	–	–
1:15,000	0.50 ml N/10 HCl	3.5	+	+	+
1:15,000	1.00 ml N/10 HCl	3.2	+	+	+
Control (no quaternary ammonium compound)	1.00 ml N/10 HCl	3.2	+	+	+
1:25,000	0	6.7	+	+	+
1:25,000	0.10 ml N/10 NaOH	9.4	+	+	+
1:25,000	0.25 ml N/10 NaOH	10.0	+	+	–
1:25,000	0.50 ml N/10 NaOH	10.2	+	+	–
1:25,000	1.00 ml N/10 NaOH	10.5	+	–	–
1:25,000	1.25 ml N/10 NaOH	10.6	+	–	–
Control (no quaternary ammonium compound)	1.25 ml N/10 NaOH	10.6	+	+	+

alkyl[C_{12}/C_{18}] isoquinolinium compounds and other heterogeneous structures.

A knowledge of the toxicologic properties of quaternary ammonium compounds is important so that the safety, health, and well-being of man and animals are not compromised by contact with these chemicals as they are manufactured, shipped, compounded, or used. Although these specific investigations are not germane, the studies to characterize the general toxicologic properties of antimicrobial quaternary ammonium compounds and the studies of the harmful consequences of these chemicals at concentrations simulating their use patterns are of primary importance. Toxicologic effect is measured at three levels: (1) at the amount necessary to bring about the response with 100% of the experimental animals, LD_{100}; (2) at the amount that will cause the response with 50% of the animals, LD_{50}; and (3) at the amount that does not produce the response with any of the animals, LD_0. The LD_{50} level is most frequently used to characterize toxicity. In addition, the methods of administering the chemical are also identified using descriptions such as oral, subcutaneous, intraperitoneal, intravenous, and dermal. Also, the toxicity is determined as an acute response following a single administration of the chemical, as a short-term or subacute response following a multiple, periodic administration of the chemical over a short period such as 30 or 90 days, and finally as a chronic response following a multiple, periodic administration over a long period, such as the animal's life span. The LD_{50} amounts of quaternary ammonium compound depend on the route of administration. A notable example of this is the work of Nelson and Lyster (1946), who examined myristal picolinium chloride and reported an LD_{50} of 250 mg/kg by oral administration, 200 mg/kg for subcutaneous injection, 7.5 mg/kg for intraperitoneal injection, and 30 mg/kg for intravenous injection. Using the acute oral LD_{50}, which is the toxicologic characteristic most frequently described, one may compare the toxicities of different quaternary compounds. When this is done with the work of Shelanski (1949) and Finnegan and Dienna (1954), we observe acute oral LD_{50} values in white rats of 445 mg/kg for alkyldimethylbenzyl ammonium chloride, 500 mg/kg for alkenyldimethylethyl ammonium bromide, 730 mg/kg for alkyldimethyl 3,4-dichlorobenzyl ammonium chloride, 420 mg/kg for diisobutylphenoxyethoxyethyldimethylbenzyl ammonium chloride, and 389 mg/kg for alkyltolymethyltrimethyl ammonium chloride. These and similar data obtained from studies with man and laboratory animals have led to the generalized conclusion that the antimicrobial quaternary ammonium compounds examined to date exhibit similar toxicologic and pharmacologic properties.

Table 13–20. *Critical Killing Dilution (10-Minute Contact Period) Required for Aqueous Dilutions of Quaternary Compounds to Kill Spores of* Trichophyton Interdigitalis; *Dilutions are Based on Anhydrous Germicide*

Chemical Structure	*Avg. Killing Dil.*
40% methyldodecylbenzyltrimethyl ammonium chloride, 10% methyldodecylxylylene bis (trimethyl ammonium chloride)	1/2000
25% *n*-alkyl (60%C_{14}, 30%C_{16}, 5%C_{12}, 5%C_{18}) dimethylbenzyl ammonium chloride, 25% *n*-alkyl (68%C_{12}, 32%C_{14}) dimethylethylbenzyl ammonium chlorides	1/800
Octyldodecyldimethyl ammonium bromide	1/5000
Didecyldimethyl ammonium chloride	1/4750

Dermal toxicity was also examined by Shelanski (1949) and Finnegan and Dienna (1954), who concluded from animal and human testing that 0.1% was the maximum concentration of the aforementioned compounds that would not produce primary irritation on intact skin or act as a sensitizer. The conjunctival mucous membrane obviously requires special consideration, which was undertaken by a number of investigators. Nelson and Lyster (1946) observed that a 1:3000 (333 ppm) solution of myr-

istal picolinium chloride produced a slight irritation of the conjunctival mucosa of rabbits. Walter (1938) reported that a 1:1000 dilution (1000 ppm) of alkyldimethylbenzyl ammonium chloride instilled into the eyes of human subjects produced burning and stinging reactions, whereas a 1:5000 dilution (200 ppm) produced no unpleasant sensations. Whitehill (1945) determined that the maximum nonirritating concentrations of alkyldimethylbenzyl ammonium chloride and cetylpyridimium chloride in the eyes of rabbits were 1:3000 (333 ppm) and 1:2000 (500 ppm), respectively. Finnegan and Dienna (1954) observed comparable irritation levels for diisobutylphenoxyethoxyethyldimethyl benzyl ammonium chloride and for alkyltolymethyltrimethyl ammonium chloride (Shelanski, unpublished); in Shelanski's investigation of laurylisoquinolinium bromide, he observed that the compound at 0.5% produced moderate to severe corneal involvement of the rabbit eye, which cleared up in 5 days. However, rabbit eyes similarly exposed and washed at 2 or 4 seconds after instillation produced only a slight conjunctival involvement, which cleared up after 2 days. A most important toxicologic consideration arising from the widely encountered application area of sanitizing food contact surfaces is the cumulative effect in animals and man of the ingestion of food contacted by the small residues of the quaternary sanitizer. The laboratory study simulating this situation is the daily oral administration of quaternary ammonium compound in the food or drinking water of test animals over a considerable period of time, including the normal life span of the animal. Such studies were made early by Nelson and Lyster (1946), who determined that myristal picolinium chloride at 0.1% in the diet of rats did not interfere with their normal growth rate, but this concentration produced death when administered as a sole source of fluid and required reduction to 0.05% to permit a normal growth rate of the test animal.

Fitzhugh and Nelson (1948) summarized the results of a 2-year chronic feeding study of rats on a diet containing 0.5, 0.25, 0.125, or 0.063% alkyldimethylbenzyl chloride; they found that all the quaternary compound concentrations were toxic to rats, as evidenced by lower growth rates over an untreated control group and as determined by the histopathologic lesions present in the treated animals. Alfredson et al. (1951), however, using a significantly larger number of experimental animals and a more randomized statistical design, demonstrated that alkyldimethylbenzyl ammonium chloride at 0.25% in the diet of rats over a 2-year feeding period did not demonstrably affect the growth, food consumption, blood picture, or histopathology of the treated animals. Dogs that were fed over a 15-week period a diet containing 0.12% or less quaternary compound demonstrated similar nontoxic effects. Shelanski (1949) noted a minimal toxicologic effect, evidenced by weight loss, a depressed growth rate, and an abnormal blood or histopathology, for dogs that were allowed a 1:5000 aqueous dilution of either alkyldimethylbenzyl ammonium chloride, alkenyldimethylethyl ammonium bromide, laurylisoquinolinium bromide, or alkyldimethyl 3,4-dichlorobenzyl ammonium chloride as their sole source of drinking water over a 6-month period, for guinea pigs administered 25, 12.5, or 5 mg/kg of the aforementioned quaternary ammonium compounds daily for a 1-year period, and for rats similarly treated daily over a 2-year period. Finnegan and Dienna (1954) demonstrated that a concentration of 2500 ppm of alkyltolylmethyltrimethyl ammonium chloride or diisobutylphenoxyethoxyethyldimethyl benzyl ammonium chloride in the diet of rats produced minimal deleterious toxicologic effects over a 2-year period, whereas a concentration of 1000 ppm produced no undesirable toxicologic effects over the same period.

The concern of the Food and Drug Administration over the unsafe adulteration of food, especially of milk, with residues from quaternary sanitizers led to label statements for these products requiring a potable water rinse following the quaternary compound sanitization and reuse of the food equipment. Recently, a careful scrutiny by the FDA of additional chronic feeding studies supplied by manufacturers of quaternary ammonium compounds has concluded that the food additive regulations should be amended to provide for the safe use of a sanitizing solution on food processing equipment and utensils and on food contact surfaces in bars and restaurants if the solution does not exceed 200 ppm of quaternary compound and if the quaternary compound solution contains equal amounts of n-alkyl [C_{12}–C_{18}] benzyldimethyl ammonium chloride and n-alkyl [C_{12}–C_{14}]dimethylethylbenzyl ammonium chloride having an average molecular weight of 384. In this instance, safe use does not require a potable water rinse of treated food contact surfaces and equipment prior to reuse (Federal Register, 1969).

A short time later, the same statement was made for an aqueous solution containing n-alkyl [$C_{12}C_{18}$] benzyldimethyl ammonium chloride having an average molecular weight of between 351 and 380 and consisting principally of alkyl groups with 12 to 16 carbon atoms with or without, but not over, 1% each of groups with 8 and 10 carbon atoms (Federal Register, 1969).

Most recently, the same statement was made for a detergent-sanitizer solution containing the first sanitizing product described as a mixture of benzyl and ethylbenzyl quaternary compounds, with the optional adjuvant substances tetrasodium ethylenediaminetetraacetate or a(p-nonylphenyl)-w-hydroxypoly(oxyethylene) (or both) having an average poly(oxyethylene) content of 11 moles (Federal Register, 1974). Gleason (1969) points out that at least 10 human fatalities implicating quaternary ammonium compounds are medically recorded as resulting from alkyldimethylbenzyl ammonium chloride solutions of 10 or 15% that were introduced into the victims via oral ingestion, intramuscular or intravenous administration, or intrauterine instillation. The reader is referred to Gleason's work for a complete description of the medical and clinical symptoms of quaternary ammonium

compound poisoning and also for the recommended treatment, including first aid, for the same. One symptom should be mentioned: namely, the ingestion of concentrated solution leads to immediate burning pain in the mouth, throat, and abdomen.

The toxicologic information presented is generalized as follows: quaternary ammonium compound germicides as concentrated solutions of 10% or more are toxic, causing death if taken internally and severe irritation to the skin and conjunctival mucosa when applied externally. With normal precautions and operating procedures, the probability of such contact is extremely remote, because the effective use-dilutions of these materials are well below the concentrated level causing death and severe injury. In addition, oral ingestion, the most likely route of accidental internal administration, is self-limiting for concentrated quaternary ammonium solutions, because the immediate burning in the mouth and throat acts as a signal. At the dilute solutions of the use levels of quaternary ammonium compound germicides, which range from several to about 1000 ppm, only the most deliberate distortions of normal operating procedures could offer acute toxicity problems. Finally, chronic toxicity, as from the cumulative effect of food adulterated with quaternary ammonium compound germicides via residues from dilute sanitizing solutions used on food processing equipment, is not a problem.

Aursnes (1982) studied the ototoxic effects of quaternary ammonium compounds (QAC), benzethonium chloride, and benzalkonium chloride, frequently used for skin disinfection. The disinfectant QACs, at concentrations of 0.1% in water solution or in 70% alcohol, were introduced into the tympanic cavity (middle ear) of guinea pigs for exposure times of 10, 30, or 60 minutes. The animals were sacrificed 2 or 9 weeks after the exposure, and the organ of Corti and vestibular neuroepithelia were studied as surface preparations with phase contrast microscopy. It was found that most of the ears exposed to the disinfectants had suffered damage, affecting both the vestibulum and the perilymphatic and endolymphatic spaces of the cochlea of the inner ear. The extent of the damage was related both to the duration of exposure and to the length of the animal's survival after the exposure. Furthermore it was found that the tympanic cavity and the perilymphatic spaces of vestibulum and cochlea were pathologically changed.

The final report on the safety assessment of benzethonium chloride and methylbenzethonium chloride has been issued by CTFA (1985) in the journal of the American College of Toxicology. It was concluded that both compounds are safe at concentrations of 0.5% in cosmetics applied to the skin. A maximum concentration of 0.02% is safe for cosmetics used in the eye area. Chronic and subchronic feeding studies indicated little or no toxic effects for both ingredients. In clinical studies, benzethonium chloride produced mild skin irritation at 5% but not at lower concentrations. Neither ingredient is considered to be a sensitizer.

APPLICATIONS

Following Domagk's publication in 1935, a large number of application areas were developed for the quaternary ammonium compounds. Initially, they were used as an adjunct to surgery, such as in preoperative patient skin treatment, degerming the hands of the surgical team preoperatively, and disinfection of surgical instruments. Wetzel, in 1935, described the superiority of 0.1% alkyldimethylbenzyl ammonium chloride over 60% alcohol for treatment of the surgeon's hands prior to surgery. Much additional evidence was provided for the treatment of the surgeon's hands with alkyldimethylbenzyl ammonium chloride by Caesar in 1935, Walter in 1938, Naegel in 1940, and Swan et al. in 1949. In 1938, both Walter and White et al. reported that the use of alkyldimethylbenzyl ammonium chloride as a skin preparation for a patient prior to surgery reduced the incidence of wound infection. Hagan et al., in 1946, compared the activity of cetylpyridinium chloride and alkydimethylbenzyl ammonium chloride to mercurials as skin preparation prior to surgery and found the quaternary compounds superior. However, Miller et al., in 1943, offered evidence that quaternary compounds applied to the skin formed a continuous residual sheet, beneath which bacteria survived.

Rahn (1946) proposed that the film is formed as an oriented adsorption of the quaternary compound on an organophilic surface, with the nontoxic ends directed toward the skin and the germicidally active ends directed toward the outside, which explained the favorable results obtained for quaternary compounds in many studies. Blank and Coolidge, in 1950, rejected the film concept and proposed instead that treatment with the quaternary compounds altered the charge of the skin to positive, which attracted and held the negatively charged bacteria, leading to the favorable, if inaccurate, results obtained in basin tests with quaternary compounds. In the same publication, they speculated that phospholipids present on the surface of the skin inhibited the antimicrobial activity of the quaternary compound.

These negative comments and the widespread popular use of hexachlorophene-based products should have seriously curtailed the use of quaternary compounds as skin degerming agents. Thus it is interesting to note that King and Zimmerman, in 1965, reporting on skin degerming practices in hospitals, did determine that 37% of hospitals were using quaternary compounds, compared to the 48% observed by Price in 1948. Walter, in 1965, described a hand degerming treatment for the surgeon as a mechanical soap hand scrubbing followed by application of a mixture of isopropyl alcohol, alkyldimethylbenzyl ammonium chloride, and cetyl alcohol. Verdon, in 1961, tested a number of skin degerming agents used in British hospitals and demonstrated the utility of quaternary compounds.

Caesar and Schmidt in 1935 and Walter in 1938 recommended the use of alkyldimethylbenzyl ammonium chloride for the disinfection of surgical instruments, Cae-

sar specifying a 1:100 concentration of quaternary compound for a 10-minute contact period and Walter a 1:1000 concentration over a 30-minute contact period. Hornung (1935) recommended the addition of sodium carbonate to the quaternary solution to prevent rusting of the instruments; Post (1946) later proposed sodium nitrite for this purpose. The early investigators, with some few exceptions, did indeed subscribe to the idea of a total spectrum of germicidal activity for the quaternary compounds and thus endorsed them as surgical instrument sterilizers. Early exceptions were Zeissler and Gunther (1939), who pointed out that Zephirol would not kill spores from the soil.

Sommermeyer and Frobisher, in 1952, using glass rods inoculated with sputum containing tubercle bacilli to simulate contaminated thermometers, demonstrated that aqueous solutions of alkyldimethylbenzyl ammonium chloride and cetylpyridinium chloride at 1:1000 dilution did not kill the organism after a 10-minute contact period, whereas tinctures of the same compounds (50% alcoholic) did in fact kill the organism. The subsequent use of specific quaternary compound neutralizers as a requisite for testing has prevented additional instances of residual bacteriostasis that were interpreted as bactericidal activity, as in the case of tuberculocidal and sporicidal testing.

The inability of quaternary compounds to kill the tubercle bacillus or bacterial spores should seriously limit their use as surgical instrument sterilizers in the future. However, in some few special cases, they are still recommended for the disinfection of comparable devices (Kasper and Kirk, 1972).

The widespread use of quaternary ammonium compounds for environmental disinfection of floors, walls, and equipment surfaces in hospitals, nursing homes, public places, and gymnasia required the development of a detergent compatible with the quaternary compounds that would permit the combination of the two operations of cleaning and disinfection. Rahn and Eseltine (1947) pointed out the utility of such a system and indicated work in progress as described by Guiteras and Shapiro (1946), who combined cetyldimethylethyl ammonium bromide with the nonionic wetting agent alkylated aryl polyether alcohol and inorganic salts. The formulation prepared by Guiteras and Shapiro diluted as 1 oz/gal aqueous solution cleaned glass slides contaminated with heavy artificial soil inoculated with bacteria. When the contact period of the slide and diluted formulation was extended to 10 minutes, all the bacteria on the soiled slides were killed, as demonstrated by negative broth subcultures of the slides and by zero-count agar plates prepared from the broth subcultures.

The concept of a one-step operation to produce cleaning and antimicrobial activity, which made the treatment of large surface areas practical, met with success and furthered research to the present time toward the development of quaternary nonionic formulations with optimum cleaning and antimicrobial properties (Greene and Petrocci, 1980). Publications (Kundsin and Walter, 1961; Lenhart et al., 1961) of both in-vitro laboratory tests and of field tests under actual conditions attest to the utility of these products. Treatment of large areas of environmental surface is accomplished by a mop-and-bucket operation, by mechanical flooding and vacuum pick-up, by sponge or cloth hand-wipe operation, and by liquid spray. As an additional treatment procedure for disinfection of surfaces, there is available liquefied gas, quaternary ammonium compound germicidal formulation discharges from pressurized containers as an aerosol spray that, according to Fulton et al. (1948), killed both *Escherichia coli* and *Staphylococcus aureus* as air-borne suspensions and surface contaminations. The patent issued to Taylor and Prindle (1966) is an instance of popular and widely used surface disinfectant and space deodorant aerosol spray composition.

Recently, treatment of surfaces is reported via a mist of quaternary ammonium compound germicide generated from commercial fogging devices that produce droplets of approximately 50 μm in diameter by the shearing action of air pressure on the liquid germicide solution. This procedure is suggested as an adjunct for terminal disinfection of hospital areas. The germicidal results obtained with 0.2% active aqueous solution of a 50:50 mixture of alkyldimethylbenzyl ammonium chloride and alkyldimethylethylbenzyl ammonium chloride in field tests and in simulated in-use tests are described by Friedman et al. (1968) and Hauser et al. (1963).

Treatment of food contact surfaces, i.e., those found on equipment in the food processing industries and on food and beverage utensils in eating and drinking establishments, in order to obtain sanitary conditions that will prevent transmission of disease through the food, is defined as a sanitizing treatment or as sanitation of such surfaces. Practicable sanitizing requires that any treatment residual will not in itself be harmful; it does not require a destruction of all the bacteria present on the treated surface. The utility of quaternary ammonium compounds in sanitizing such surfaces was suggested by Krog and Marshall (1940), who experimentally contaminated the rims of glass drinking tumblers, allowed them to air dry, and treated them with a 1:5000 aqueous dilution of alkyldimethylbenzyl ammonium chloride for various contact periods before swabbing and plating procedures were employed to enumerate the viable bacteria present; this demonstrated a reduction to less than 100 bacteria per rim after only a 1 minute contact period. Two years later they reported that the use of alkyldimethylbenzyl ammonium chloride at a 1:6000 dilution in a dairy pasteurizing plant caused a 60 to 98% reduction of the bacteria count on the milk handling and processing equipment (Krog and Marshall, 1942). Frayer (1943), Mueller et al. (1946), DuBois and Dibblee (1946), Puhle (1950), and Speck and Lucas (1957) are several of the many investigators who determined the utility of quaternary ammonium compounds as germicides for the dairy farm and dairy plant. Similar reports to demon-

strate the advantage of quaternary compounds as germicides in other food processing industries have appeared, such as those of Penniston and Hedrick (1945) in the egg processing industry, Tressler (1947) in the fishing industry, Lehn and Vignolo (1946) and Resuggan (1951) in the brewing industry, and Casey (1972) in the sugar refining industry.

The treatment of large amounts of water to destroy and prevent the proliferation of disease-producing microorganisms, of industrial process-interfering microorganisms, and finally of noxious, esthetically undesirable microorganisms is an important application area of the quaternary ammonium compounds. The destruction of cysts of *Entamoeba histolytica* was reported by Fair et al. (1945) and by Kessel and Moore (1946). Kessel and Moore offered their opinion that quaternary ammonium compounds should be considered for the emergency sterilization of drinking water; this opinion was based on the high germicidal levels observed. Sotier and Ward (1947) observed that a product containing 0.5% of Hyamine 1622 was an effective disinfectant of new water mains and of hemp used to prepare joints on the mains. Quaternary ammonium compounds have been recommended for treating process water in paper mills (Erskine et al., 1958; Shema, 1966) to prevent the proliferation of microorganisms on the surface of equipment contacting the pulp solution. These proliferations form microbial slimes that become detached and are carried along to become spots in the paper, often causing paper breaks during the manufacturing process that result in expensive, time-delaying machine shutdowns. Quaternary compounds are used in cooling water systems (Darragh and Stayner, 1954; Berneschot et al., 1964) to prevent proliferation of bacteria in the circulating cooling water system that would form microbial slimes that may reach the heat exchange units and impair their operational efficiency. Prusick and Gregory (1956) recommended the use of dicocodimethyl ammonium chloride to prevent proliferation of bacteria in injection water or brine used in secondary oil recovery operations. The injection water is pumped under pressure into injection wells drilled around the producing well, so that as it spreads through the subterranean sand structures containing residual oil, it will displace the residual oil in the direction of the producing well and increase the productivity of the well. If there is a proliferation of microorganisms in the injection waters, the subterranean sand structures will become plugged, requiring greater pressures to be applied to the injection waters until the operation cannot function. Steinberger (1963) described the ability of N-alkyl crude tar base quaternary ammonium compounds at 5 to 20 ppm to inhibit the proliferation of *Desulfovibrio desulfuricans*, which with pseudomonas species are primarily involved in plugging the sand formation.

Outdoor swimming pools are regularly treated with quaternary ammonium compounds to prohibit growth of algae, an esthetic rather than a public health problem. Palmer and Maloney (1955) determined that 2 ppm of several different quaternary ammonium compounds did effectively inhibit the growth of various algae species. Antonides and Tanner (1961) recommended a formulation containing trimethylalkyl ammonium compound at 4.5 ppm as a swimming-pool algicide and bactericide. Shay et al. (1964) demonstrated that alkyldimethylisopropylbenzyl ammonium chloride at 7 ppm killed both *Escherichia coli* and *Streptococcus faecalis* at a 30-second contact time by the AOAC test method prescribed for swimming pool bactericides.

The positively charged functional portion of the quaternary molecule is attracted to and substantive to negatively charged fabric; it may be applied to the fabric from a quaternary solution by rinsing, padding, or spraying. Taking advantage of this phenomenon, Benson et al. (1947, 1949) proposed that diapers be treated with [methylbenzethonium chloride]p-diisobutylcresoxyethoxyethyldimethylbenzyl ammonium chloride monohydrate in the final rinse of the laundry cycle. Treated diapers would prevent the formation of ammonia from urine by the action of ammonia-producing bacteria found in the infant's feces; thus, ammonia dermatitis leading to diaper rash could be prevented. Lawrence (1950) pointed out the need to apply chemical treatment between the laundry loads of different customers using public automatic clothes-washing machines.

Although large commercial laundries could provide sufficient volumes of hot water at disinfecting temperatures, the small automatic installations could not. Lawrence used alkyldimethylbenzyl ammonium chloride to treat laundry in the final rinse cycle of automatic machines in a coin-operated self-service laundry. He determined that there were high bacteria counts in the first soak and wash cycle water, which following treatment with the quaternary compound at 1:5000 (200 ppm) or 1:10,000 (100 ppm) dilution, were reduced to zero counts in the deep rinse cycle.

McNeil and Choper (1962) treated naturally soiled wash loads at the warm or hot water setting and in the wash or rinse cycle with alkyldimethylbenzyl ammonium chloride to provide 200 ppm of active quaternary compounds in the presence of anionic or nonionic laundry detergent. Compared to untreated wash loads, the quaternary compound used with the warm or hot water setting consistently reduced the numbers of bacteria in the wash and rinse waters and on fabric swatches attached to laundry items. This occurred with the quaternary compound added to the rinse cycle, and to the wash cycle when a nonionic detergent was used. Utilizing the AOAC antimicrobial laundry additive test procedure (Petrocci and Clarke, 1969), a simulated use test, Shay and Petrocci (1968) demonstrated sanitization of fabric during the laundry cycle following addition of a 50:50 mixture of alkyldimethylbenzyl ammonium chloride and alkyldimethylethylbenzyl ammonium chloride at a 200 ppm level based on the weight of dry laundry fabric. In addition, fabric similarly treated and allowed to air dry demonstrated residual bacteriostatic activity against

Staphylococcus aureus, *Escherichia coli*, and *Brevibacterium ammoniagenes*, and also demonstrated residual self-sanitizing activity against *Staphylococcus aureus* and *Escherichia coli*. Some of the miscellaneous applications for quaternary ammonium compounds are as preservatives. Lawrence (1950) evaluated a large number of chemicals as preservatives for ophthalmic solution and selected benzalkonium chloride at 1:5000 and 1:10,000 as superior. More recently, Stark (1985) used Polymeric quaternary ammonium compounds, namely Polyquat, for disinfecting and preserving solution for contact lenses, ointments, and other ocular medicaments. The polyquaterium-1 (Onamer M) has been approved by the FDA as a preservative in several brands of contact lens soaking solutions at 0.01 to 0.001% [Opti-Soft, Opti-Clean, Opti-Tears, Opti-Free, and Tears-Natural II]. This product has replaced benzalkonium chloride and Thiomerosal in these preparations. Bernarducci and Harrison (1988) have discovered that water-soluble cationic polymers (Busan 77, WSCP, Onyxsperse 12S, Onamer M), when formulated in an aqueous medium with one or more nonionic surfactants, provide stable, isotropic liquid laundry detergent and sanitizer compositions having good detergency and bactericidal properties, and in addition are less irritating to the eye and practically nonirritating to the skin.

Like et al. (1975) examined a number of compounds, including a 50:50 mixture of alkyldimethylbenzyl ammonium and alkyldimethylethylbenzyl ammonium chlorides, as preservatives for cosmetic oil-in-water systems using amine oxides as emulsifiers. At 1000 or 2000 ppm, the quaternary ammonium compounds reduced the microbial count of the cosmetic system inoculated with bacteria and fungal spores from an initial count of several million per ml to less than 10 per ml over an 8-week incubation period, including a reinoculation after 4 weeks of incubation.

In another instance, as a fungistat for exterior latex paint films, Ramp et al. (1966) demonstrated that alkyldimethyl ethylbenzylammonium cyclohexylsulfamate at about a 1% level in exterior latex paints would produce a paint film resistant to attack by fungi. In another instance, as a moth-proofing agent for wool fabric, Tolygesi et al. (1971) concluded that tricaprylylmethyl ammonium chloride was superior to all other quaternary ammonium compounds tested for this purpose. Both compounds applied to wool fabric at 0.15 to 0.40% add-on levels protected the fabric from the black carpet beetle and the webbing clothes-moth larvae.

MODE OF ACTION

The earliest accounts of the quaternary ammonium compounds related antimicrobial activity to chemical structure by utilizing homologous series of quaternary compounds and noting the effect on antimicrobial activity offered by variations in structure (Jacobs, 1916). This method of collecting information on the antimicrobial attributes of a quaternary compound is constantly employed to determine the most effective structure that may be used as an item of commerce. Beyond this purpose, this method contributes little to our understanding of the basic mechanism of quaternary ammonium compounds as antimicrobial agents. Although the mode of action has not yet been completely or definitively described in detail, there are well defined and accepted steps explaining the mode of action of cationic disinfectants or antiseptics as reported by Ikeda and Tazuke (1985) and Franklin and Snow (1989). Many techniques have been used in elucidating the mode of action of antimicrobial agents. Once the primary site of action is established the overall effect of the drug on the metabolism of microbial cells can often be explained. The most important site of adsorption is the cytoplasmic membrane. Spheroplasts or protoplasts lacking the outer cell wall layers will bind the cationic antiseptic and may be lysed or damaged. Adsorption by isolated cell membranes can be demonstrated.

The extent of killing of the bacteria is governed by five principal factors: (a) Concentration of antiseptic or disinfectant; (b) nature of bacterial cells and density; (c) time of contact; (d) temperature of medium; (e) the pH; and (f) the presence of foreign matter.

The adsorption of a given amount of the compound per cell leads to the killing of a definite fraction of the bacterial population in the chosen time interval. The lowest concentration of the antiseptic that causes death of the bacteria also brings about leakage of cytoplasmic constituents of low molecular weight. The most immediately observed effect is loss of K^+ ions. The increased permeability is a sign of changes in the membrane that are initially reversible but become irreversible on prolonged treatment. The necessary characteristic of an antiseptic is its bactericidal action, but there is often a low and rather narrow concentration range in which its effect is bacteriostatic. At this low concentration, certain biochemical functions associated with the bacteria membrane may be inhibited. In the presence of a higher concentration of antiseptic and after prolonged treatment, the compound usually penetrates the cell and brings about extensive ill defined disruption of normal cellular functions. The primary effect of these antiseptics on the cytoplasmic membrane is thus established beyond doubt, but secondary actions on the cytoplasmic processes are less defined and may vary from one compound to another.

A review of the most reliable evidence on the mode of action of cationic antiseptics suggests the following generalizations:

1. Adsorption of compound on the bacterial cell surface
2. Diffusion through the cell wall
3. Binding to the cytoplasmic membrane
4. Disruption of the cytoplasmic membrane
5. Release of K^+ ions and other cytoplasmic constituents

6. Precipitation of cell contents and the death of the cells

It is well known that the bacterial cell surfaces are usually negatively charged, and then that adsorption of polycations on to the negatively charged cell surfaces (process 1) is expected to be enhanced with increasing molecular weight of the polymer due to the increasing charge density of the polycations. The binding of polymers to the cytoplasmic membranes and its disruption is expected to be facilitated by increasing the molecular weight of the polymer and by increasing the amount of the bound polymers to the bacteria cells. To examine this hypothesis Ikeda and Tazuke (1985) studied the effect of MW of the polymers on lysis of protoplasts, which are bacteria cells freed entirely of cell walls, so that the interactions processes 1 (adsorption) and 2 (diffusion) of the polymer with the protoplasts can be left out of consideration. The amount of cytoplasmic constituents released from protoplasts and from intact cells of *Bacillus subtilis* was measured at 260 nm after exposure to fractionated samples of polymers of various MW. Lysis of protoplasts was clearly enhanced with increase in MW of polymer[poly{n-butyldimethyl[vinylbenzyl]-ammonium chloride]}. A bell-shaped curve relationship of the activity to the MW of fractionated polymer was observed in the case of intact cells of *B. subtilis*. Because separate experiments have shown that release of cytoplasmic constituents from intact cells correlates well with the death of the cells, these results support the concept that the mode of action of the polymeric biocides is disorganization of cytoplasmic membrane followed by rupture of membrane, leading to the death of the bacteria (Franklin and Snow, 1989).

Ikeda and Tazuke in 1985 reported an optimal MW region of 14,300 for the 6,6-ionene bromide with antibacterial activity with minimum bactericidal concentration of 6.6 ppm to 10 ppm against *Staphylococcus aureus*. The same workers reported that polymeric biquanides of MW 11,900 and poly{alkyl[C_2–C_{12}]dimethyl[vinylbenzyl]ammonium chloride} of similar MW exhibit better bactericidal action against gram-positive than gram-negative bacteria. The poly{dodecyldimethyl[vinylbenzyl]-ammonium chloride} was the most active at 0.5 ppm against *Staphylococcus aureus*, which suggests that hydrophobicity plays an important role in bactericidal action.

A primary consideration in examining the mode of action is the characterization of quaternary compounds as surface-active agents, or surfactants. These are adequately defined by James (1965) as compounds with a structural balance between one or more water-attracting (hydrophilic) groups; depending on the nature of the charge or absence of ionization of the hydrophilic group, they may be classified as anionic, cationic, or nonionic. Quaternary ammonium compounds are cationic surface agents and possess such properties as a reduction of the surface tension at interfaces upon absorption; a ready attraction to an absorption on surfaces possessing a negative charge such as wool, glass, protein, and bacteria; the formation of ionic aggregates or micelles with attendant changes in electrical conductivity, surface tension, and solubility; a precipitation, complex formation, and denaturing effect on proteins; and an inhibiting or stimulating effect on enzyme activity (James, 1946).

With these demonstrable properties for cationic surfactants, it was natural for some investigators (Cowles, 1938) to attribute the antimicrobial activity of quaternary ammonium compounds entirely to the presence and amount of surface activity. However, this obvious explanation has been refuted (Gershenfeld and Milanick, 1941; Hotchkiss, 1946).

An excellent presentation on the mode of action of cationic surface-active agents on microbial cells was given by Hugo (1965). The concepts concerning the mode of action of surface-active agents on microorganisms may be divided into five broad categories.

1. Effects on protein. See Putnam (1948) for protein-denaturing agents, Khun and Dann (1940) for enzyme-disrupting agents. Quaternary ammonium compounds are surface-active agents and will denature protein or cause dissociation of an enzyme from its prosthetic group. This effect is usually caused at concentrations much higher than those which are lethal to the microbial cells, and it is unlikely that this effect is the primary cause of the antibacterial activity of surface-active disinfectants except at high concentration.

2. Effects on metabolic reactions. See Baker et al. (1941) on the aerobic and anaerobic respiration of glucose by a variety of bacteria and Ordal and Borg (1942) on the oxidation of lactate by *Escherichia coli* and *Staphylococcus aureus*. Attempts have been made to relate inhibition of metabolism with inhibition of growth. Such a correlation has been observed at high concentration, and almost any degree of agreement or disagreement may be demonstrated depending upon the enzyme system chosen and the test organism. It can be expected that those enzymes located in the cytoplasmic membrane will be the first to be affected. Penetration of the cell will follow, and cytoplasmic enzymes will then be inhibited. The enzyme inhibition is not the primary or main lesion caused by these compounds. A specific detergent-sensitive enzyme does not exist or has not been discovered.

3. Effects on cell permeability. See Armstrong (1957) on cytolytic damage and phosphorus loss and Scharff and Maupin (1960) on membrane damage and loss of potassium. The cytoplasmic membrane is probably the organelle most sensitive to surface-active agents within the cell of bacteria and yeasts, and the alteration in the semipermeable properties of this structure can lead to leakage of metabolites and coenzymes and disturbance in the delicate balance of metabolite concentrations within the cell. This lesion may be a major contribution to the death of a cell and cause an apparent loss in enzymic activity due to the loss or dilution of coenzymes or substrates. There is a well established relationship between cytolytic

action and surface tension. This lends support to the idea that cytolytic damage may in fact be the primary lesion caused by surface-active substances.

4. *Stimulatory effect of the glycolysis reaction.* This is suggested by the work of Strickland (1956) and Bihler et al. (1961). This reaction is of interest, but because the effect is elicited at concentrations well below those which are antibacterial, it cannot be considered of significance for the antibacterial action of surface-active agents.

5. *Effect on an enzymatically-maintained dynamic membrane.* This is offered as a speculative theory by Newton (1958). Results to support this interesting hypothesis are awaited. This concept of the dynamic cell membrane enzymatically maintained could well be the detergent-sensitive enzyme, which as it was stated before does not exist.

Hugo has selected the effect on the cytoplasmic membrane controlling the cell permeability as the mode of action for quaternary ammonium compound germicides. This, he feels, is most consistent with the data presented to date, and it is well accepted (Franklin and Snow, 1989; Ikeda and Tazuke, 1985).

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